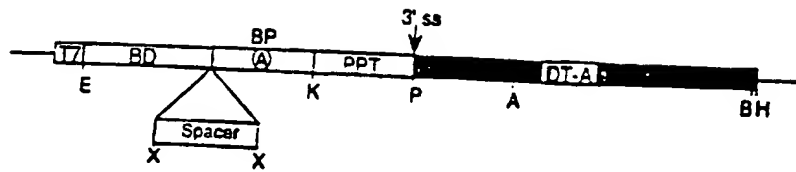


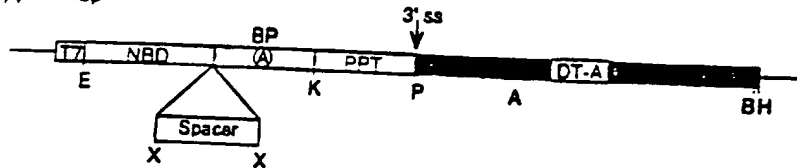
FIGURE 1A:



(B)



(2) pPTM-Sp



(C)

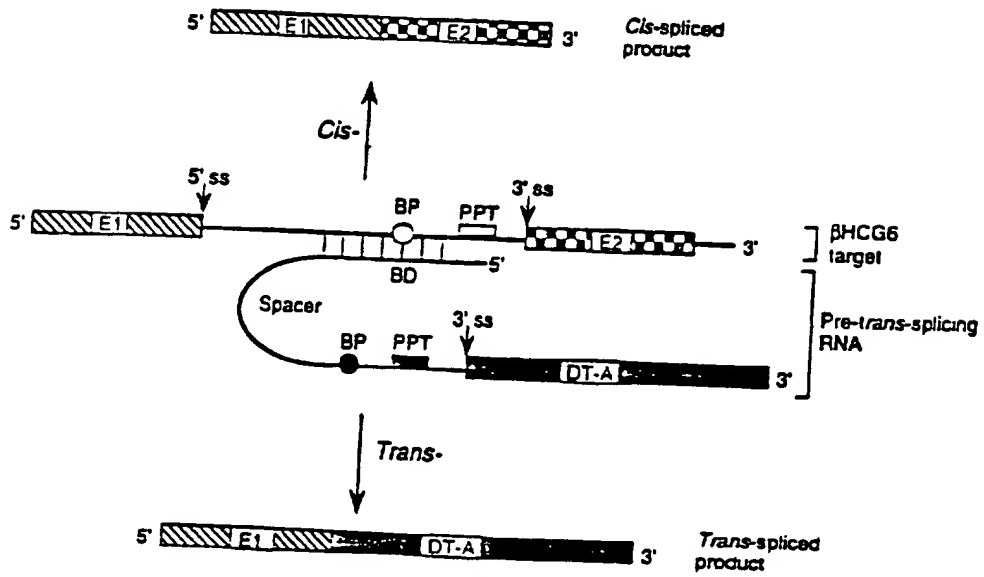
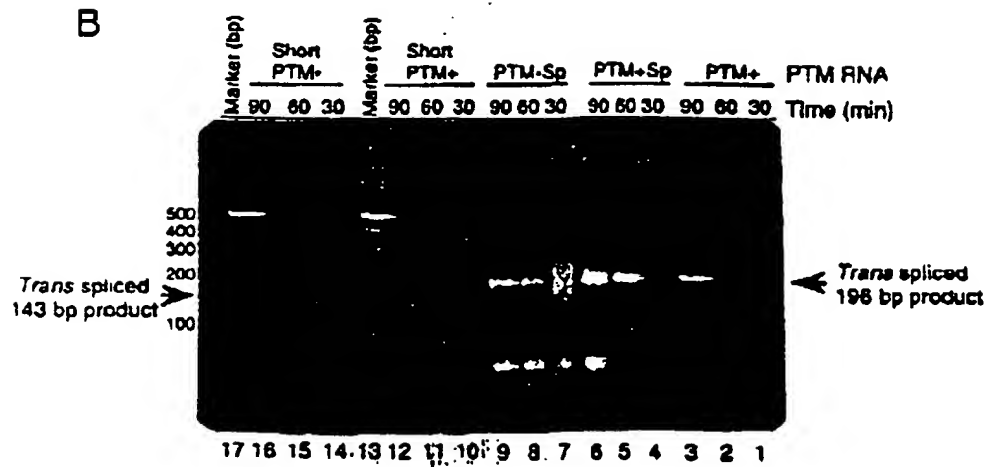
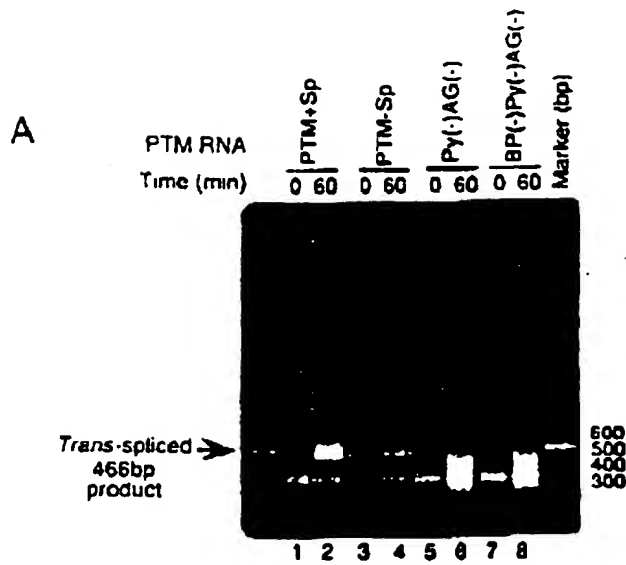


Figure 1 B-C



[The page contains extremely faint, illegible markings.]

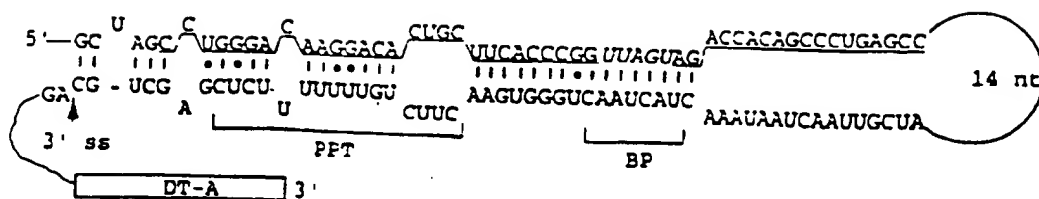
Exon 1 of BICG6

1st coding nucleotide of DT-A

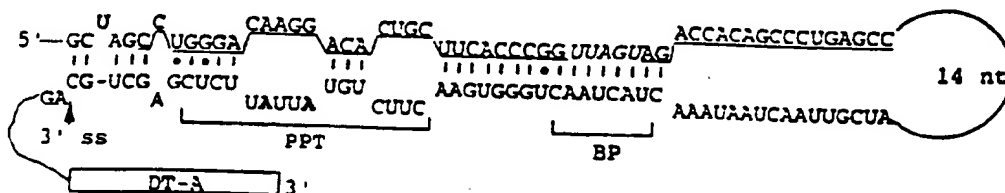
$\frac{d}{dt} \left(\frac{\partial L}{\partial \dot{x}} \right) = \frac{\partial L}{\partial x}$

(A)

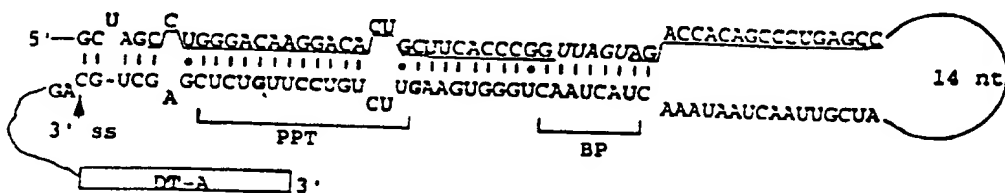
1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:



(B)

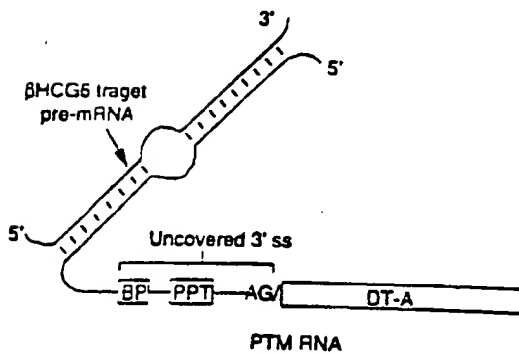


Figure 4A-B

(C)

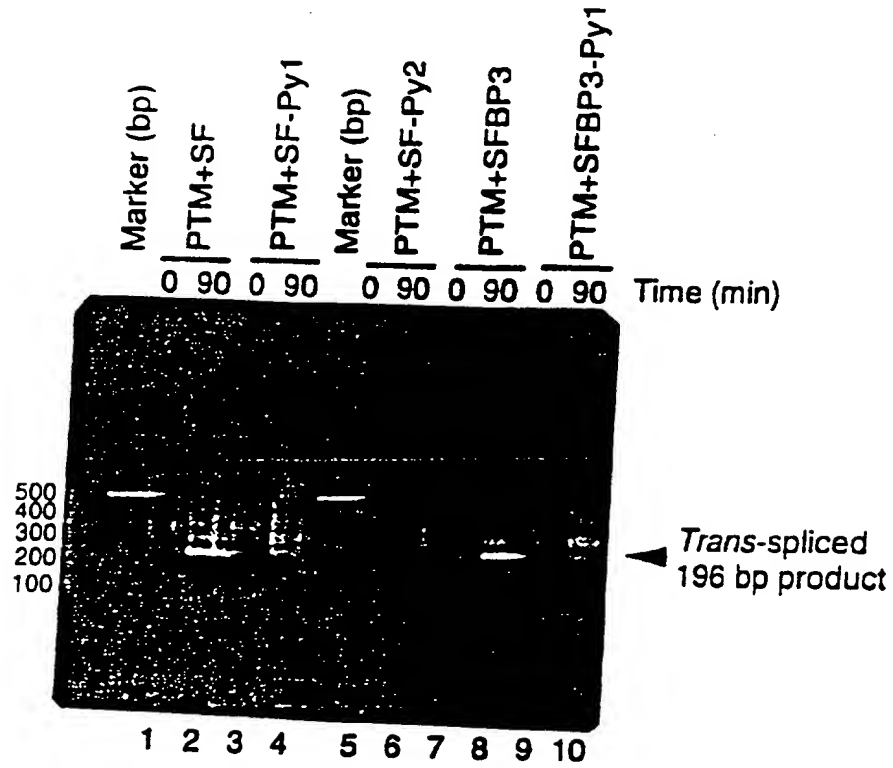


Figure 4c

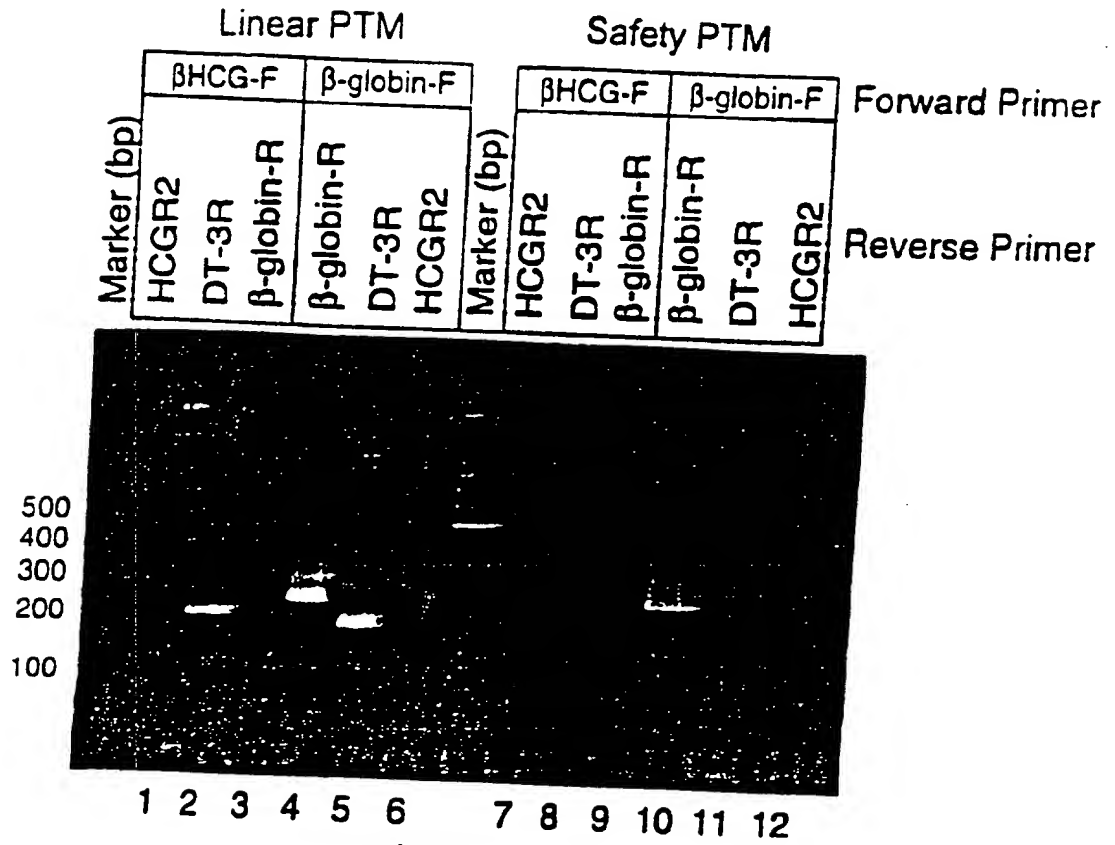


Figure 5

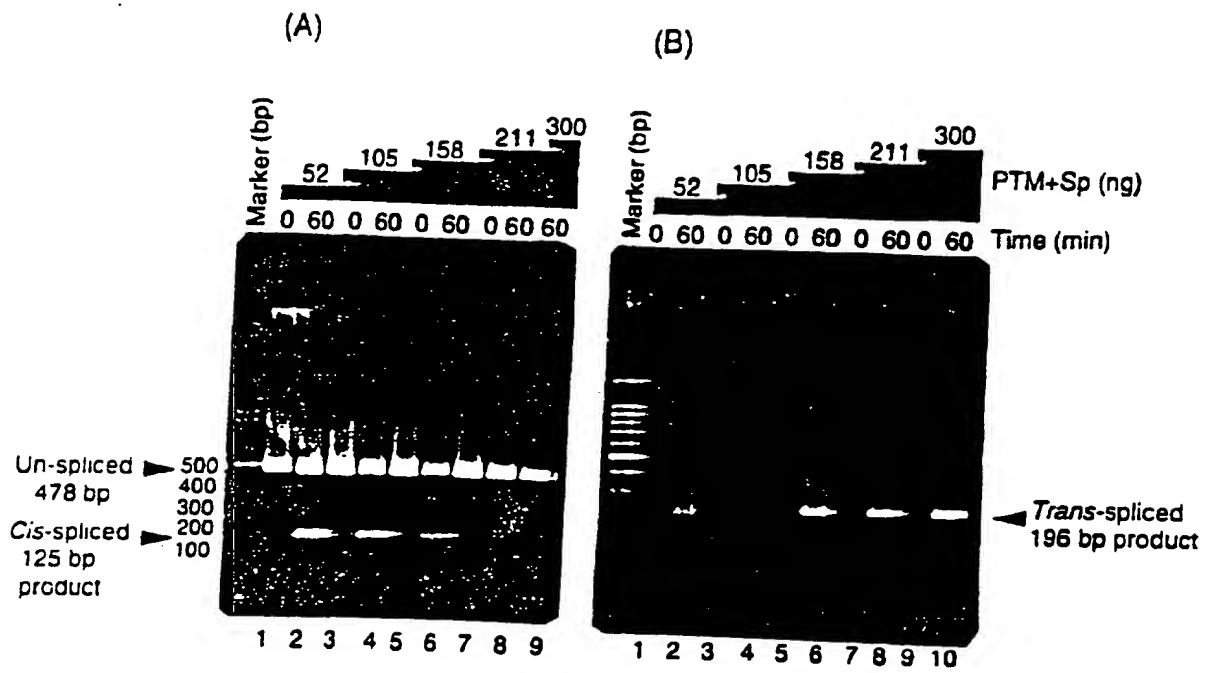
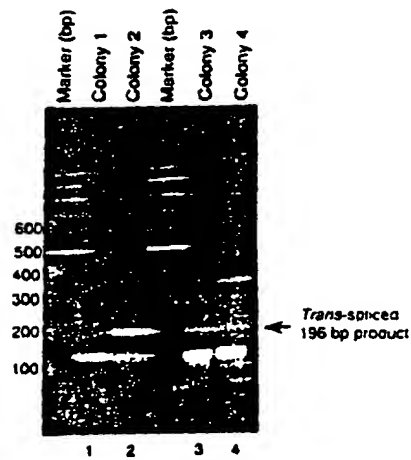


Figure 6

Figure 7

(A)



(B)

Exon 1 of β HCG6 ↓
 5'-CAGGGGACGCACCAAGGATGGAGATGTTCCAG-GGCGCTGATGATGTTGTT-
 ↑ 1st coding nucleotide of DT-A
 GATTCTTCTTAAATCTTTTGTGATGGAAAACCTTTCTTCGTACCACGGGACTA
 AACCTGGTTATGTAGATTCCATTCAAAAA-3'

Double Splicing Pre-therapeutic RNA

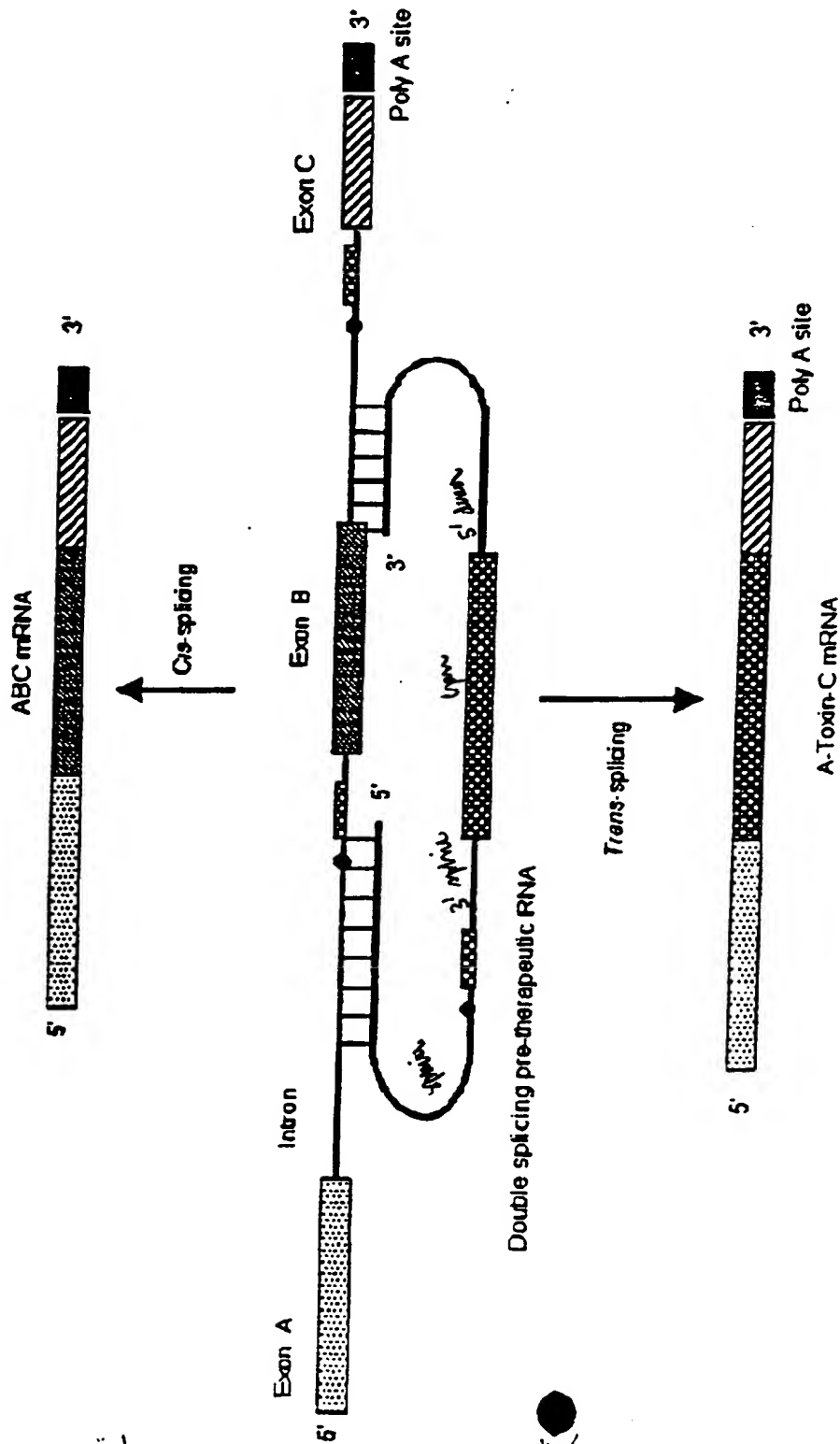


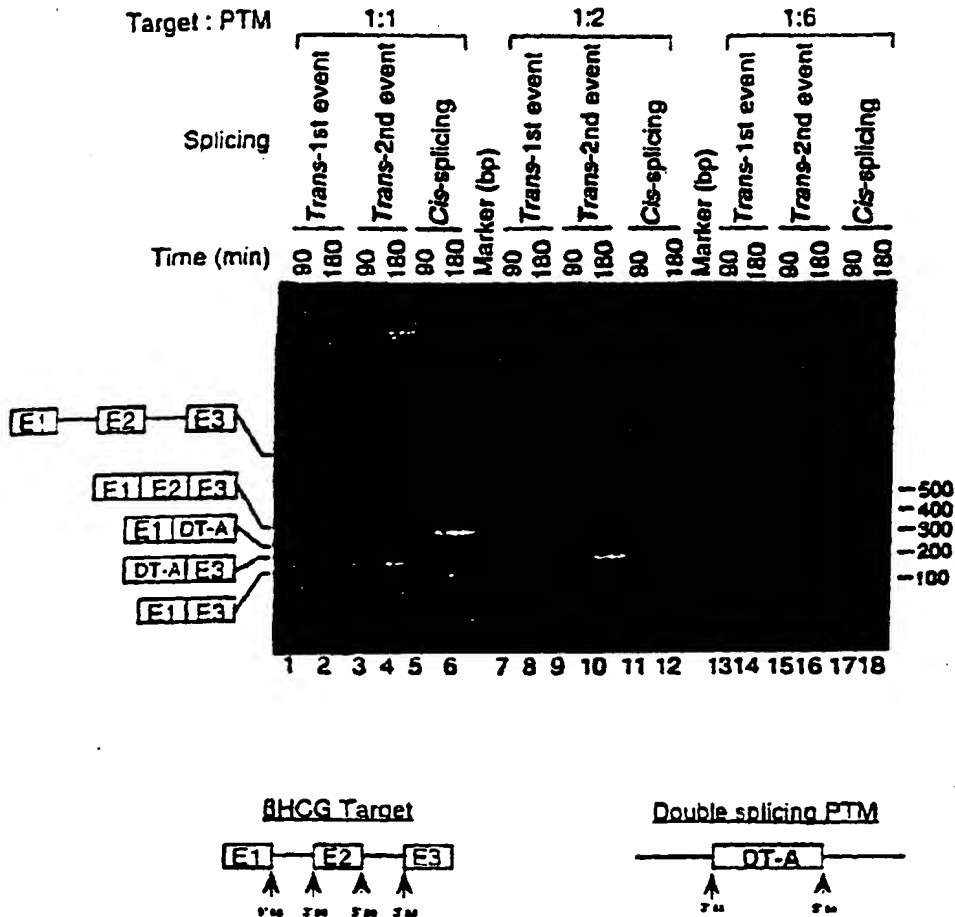
Figure 8A

31304B-A

(Sheet 12 of 58)

Selective *Trans*-splicing of a Double Splicing PTM

(3' ss of PTM to 5' ss target and, 5' ss of PTM to 3' ss of target)



Cis-spliced products

E1-E2-E3 = Normal *cis*-splicing (277bp)

E1-E3 = Exon skipping (110bp)

Trans-spliced products

E1-OT-A = 1st event, 196bp. *Trans*-splicing between 5' ss of target & 3' ss of PTM.

OT-A-E3 = 2nd event, 161bp. *Trans*-splicing between 3' ss of target & 5' ss of PTM.

Figure 8B

31304B-A
(Sheet || Of 58)

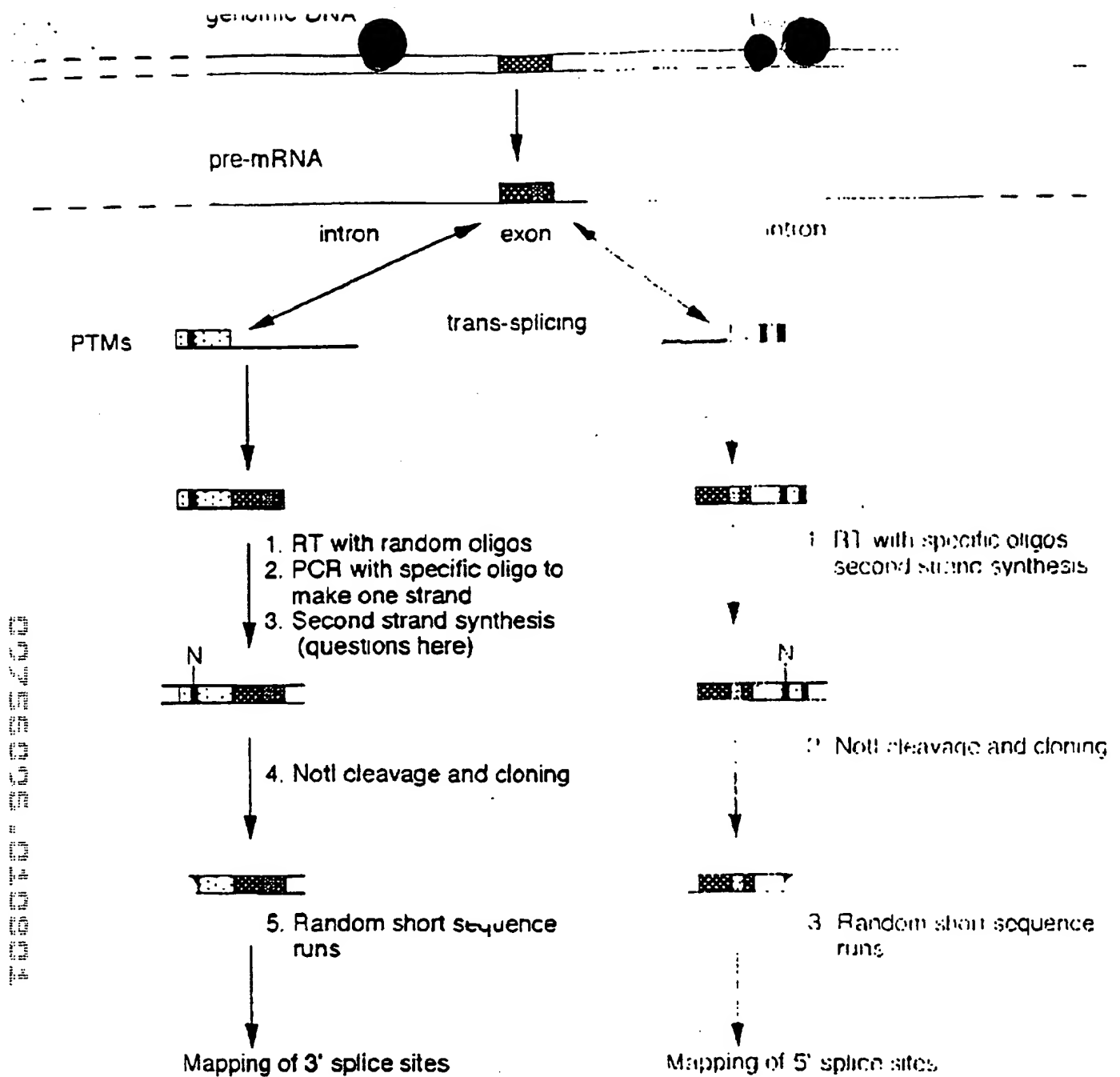


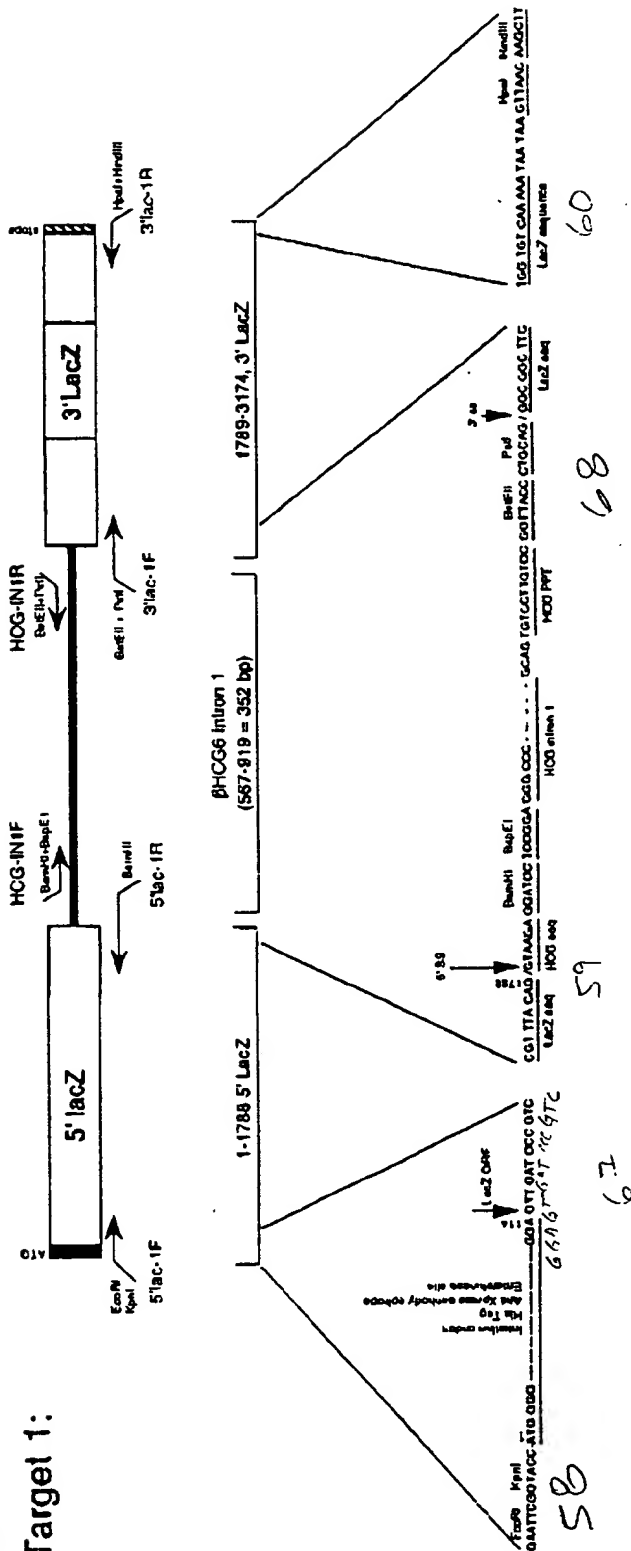
FIGURE 9

31304B-A
(Sheet 12 Of 58)

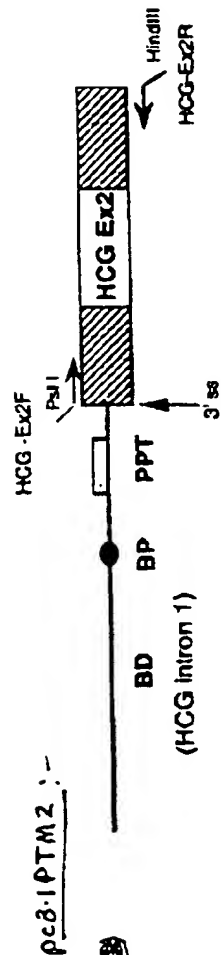
Knock Out LacZ Model Constructs

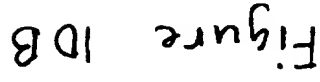
pc3.1 Lac-TI

Target 1:



PTMS



[illegible]

(187 to 191 7'40)

31304 B-A
(Sheet 15 of 58)



FIGURE 11A

[illegible]

Figure 11B

51307 15-11
(Sheet 17 of 58)



51307 15-11

FIGURE IIC

Nucleotide Sequence Demonstrating that *Trans*-splicing is Accurate

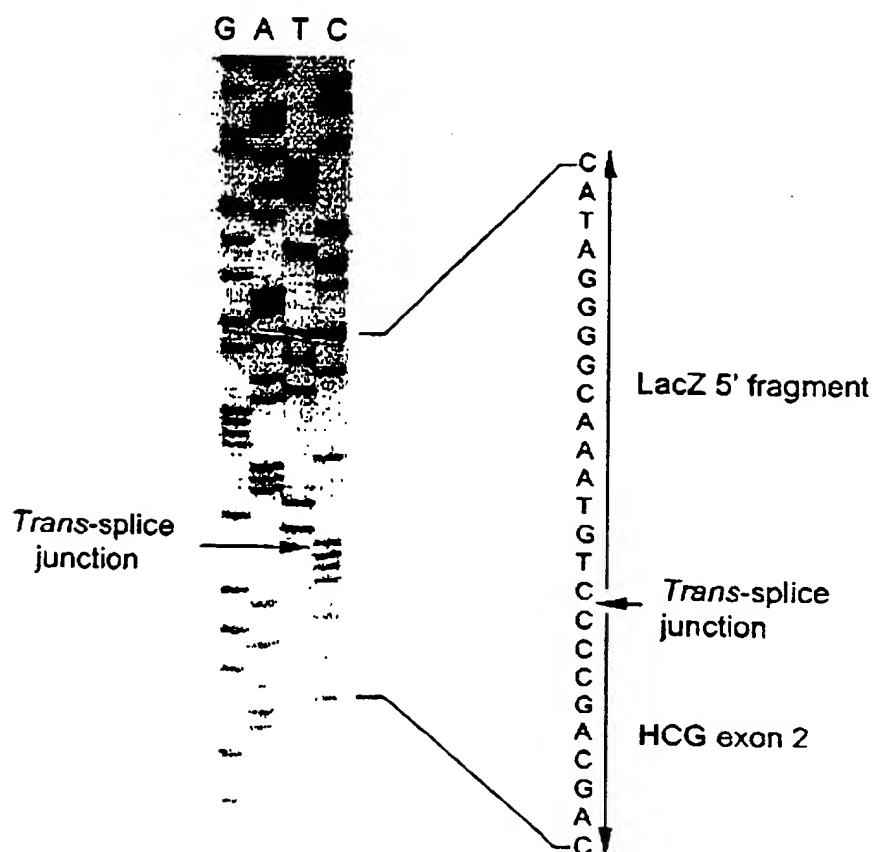


FIGURE 12 A

31304-B-A
(Sheet 18 of .58)

(1) Nucleotide sequences of the cis-spliced product (285 bp) :

BioLac-TR1

GGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAAACAGTCTTG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGCGGCTTCGTCTATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTTGGTCGGCTTACGGCGGTGATTT

Lac-TR2

TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGGCGACCGCACGCCGCATCCAG

(2) Nucleotide sequences of the trans-spliced product (195 bp)

BioLac-TR1

GGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAAACAGTCTTG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

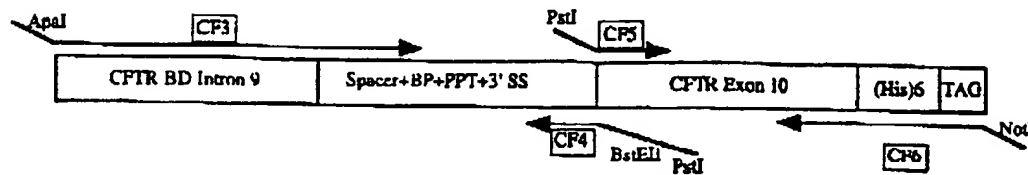
HCGR2

GAGCATGGGCGGGACATGGGCATCCAAGGAGCCACTTCGGCCACGGTGCCG

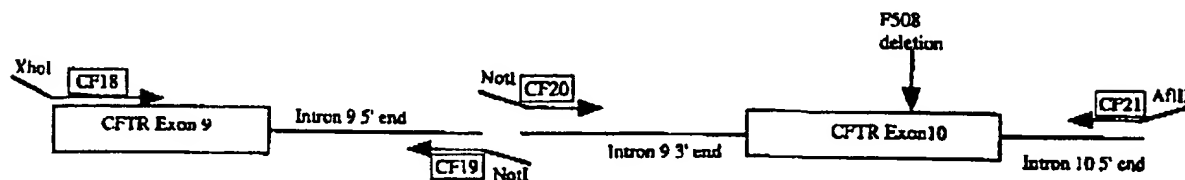
Figure 12 B

31304-B-A
(Shut 19 of 58)

CFTR Pre-therapeutic molecule (PTM or "bullet")

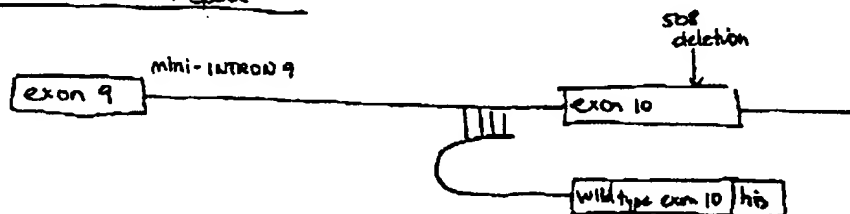


CFTR mini-gene target - Construction



TRANS-SPLICING Repair

Binding
of
PTM to TARGET



↓ splicing

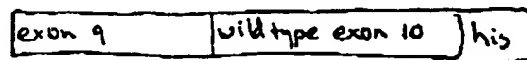
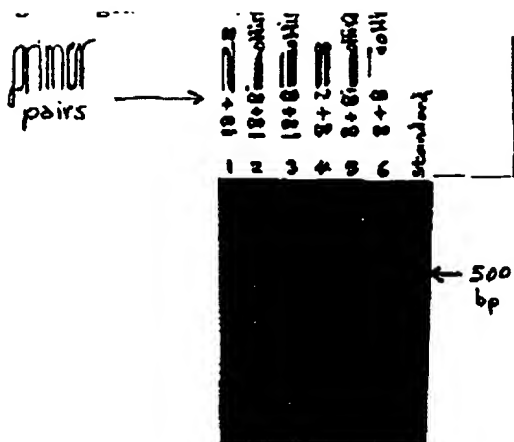


Figure 13

31304-B-A
(shut 2004.58)

Figure 14



31304 B-A
(Sheet 21 of 58)

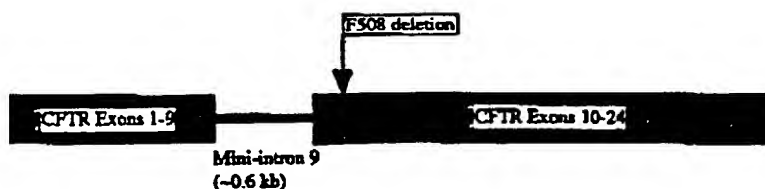
31304-A-B
(Airt 22 of 58)

EXPERIMENT 2

Repair of an exogenously supplied CFTR target molecule carrying an F508 deletion in exon 10.

PTM

+

CFTR Target (mini-gene)

Cotransfect PTM and Target molecules in HEK 293 cells and detect repaired CFTR mRNA by RT-PCR.

Repaired CFTR mRNA

Figure 1b

31304-A-B

Sheet 23 of 58

EXPERIMENT 3

Repair of endogenous CFTR
transcripts by exon 10 invasion
using a double splicing PTM

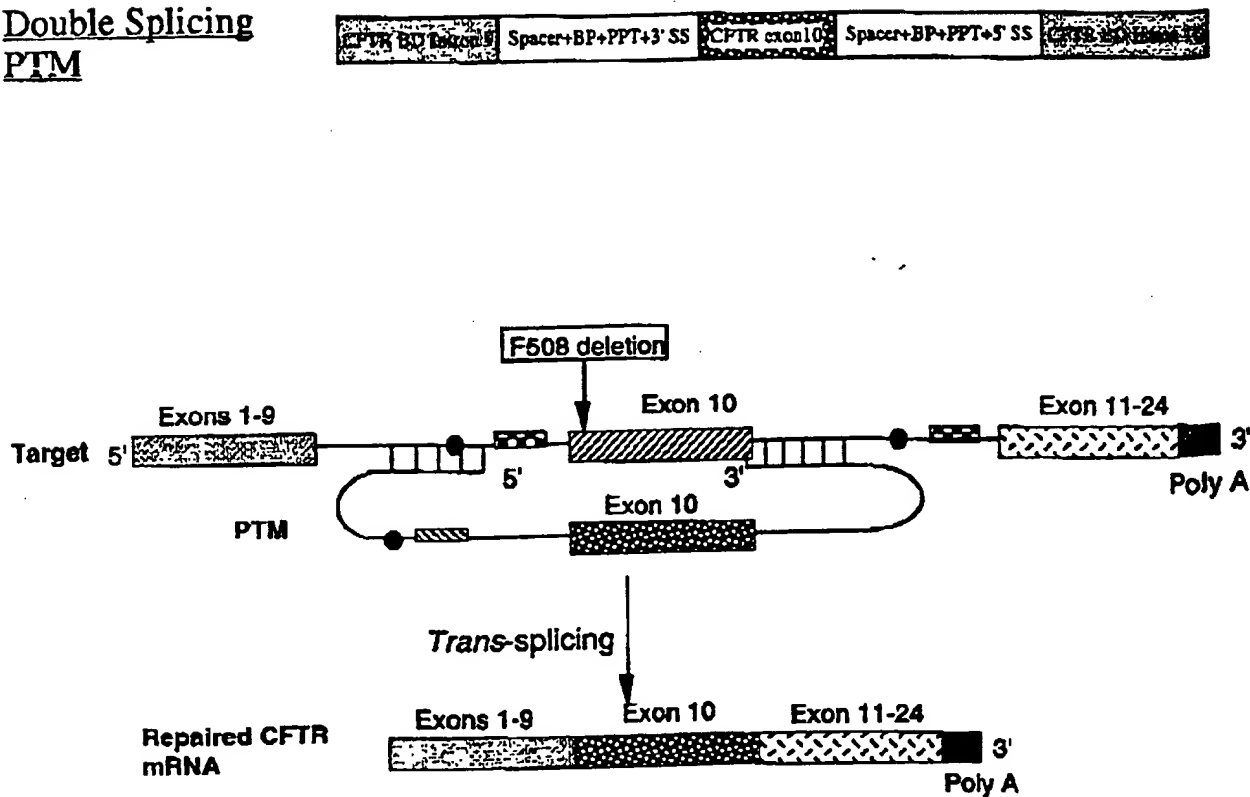
Double Splicing
PTM

Figure 17

31304 B-A

Sheet 24 of 58

Double Trans-splicing Specific Target

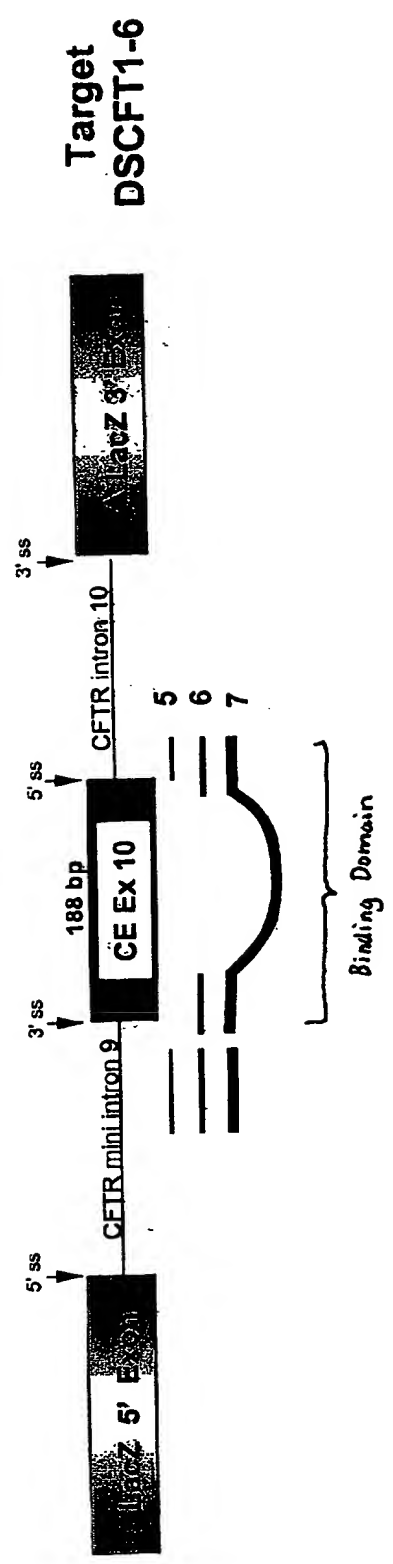
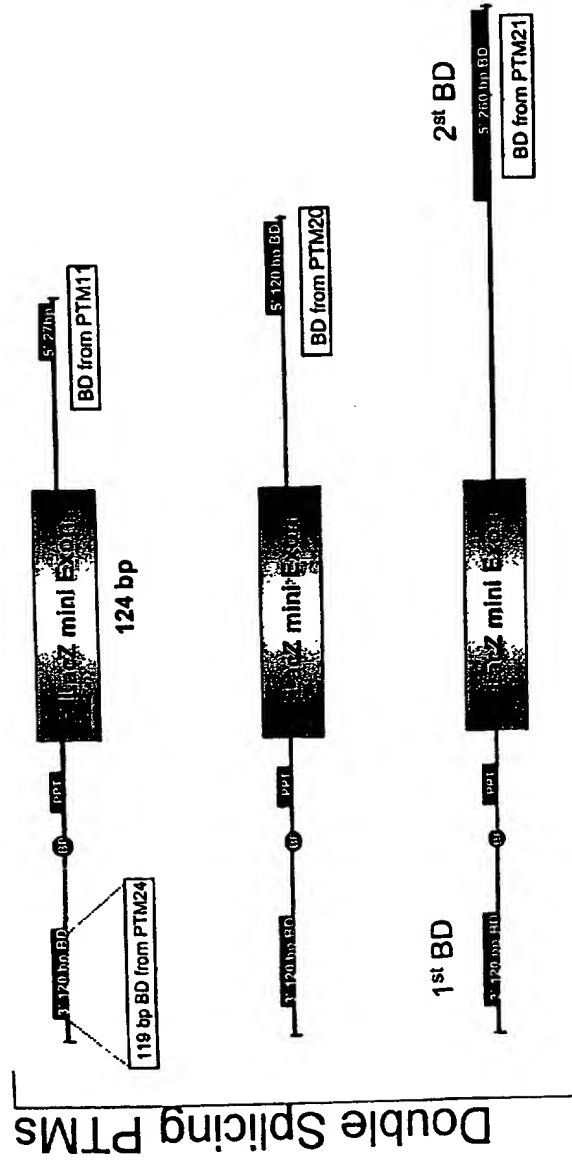


Figure 18

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Double Trans-splicing PTMs



DSPTM-5

PTM with 27 bp BD & masks 5' single splice site

DSPTM-6

PTM with 120 bp BD & masks both 5' & 3' splice sites

DSPTM-7

PTM with 260 bp BD masking both the ss & the entire CFTR Ex10

Figure 19

Double Trans-splicing β -Gal Model

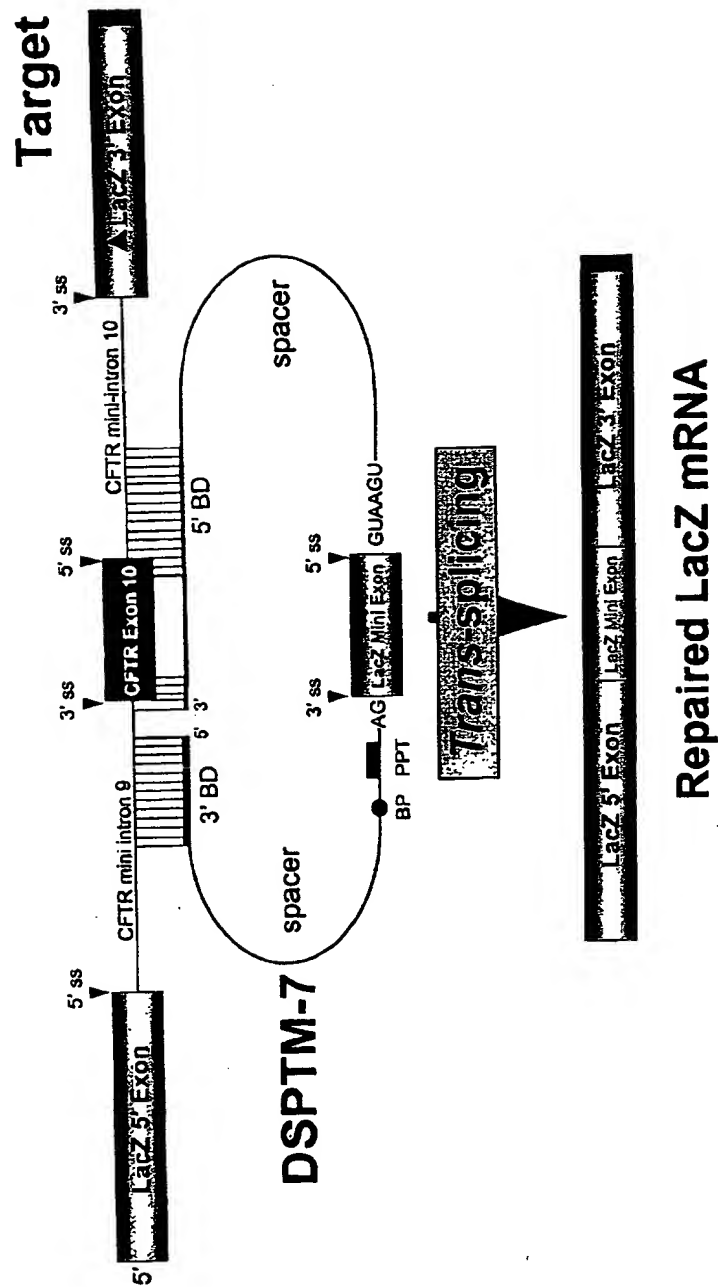
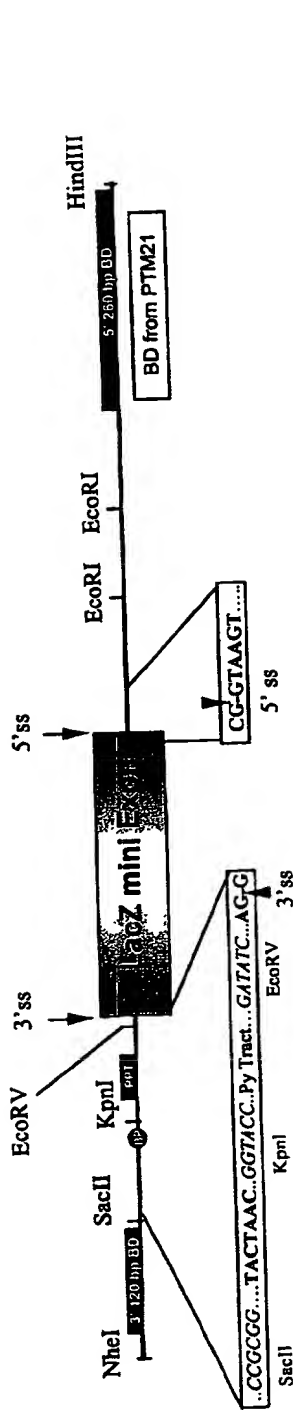


Figure 20

Important Structural Elements of DSPTM-7: (Double splicing PTM with all the necessary splice elements i.e. has both 3' and 5' functional splice sites and the binding domains)



(1) 3' BD (120 BP) : GATTCACCTTGCTCCAATTATCATCCTAAGCAGAAGTGATATCTTATTGTTAAAGATTCTATTAACATCTTTGATTC
AAAATATTTAAATACTCTCCTGTTTCATACCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTGCTCGAA

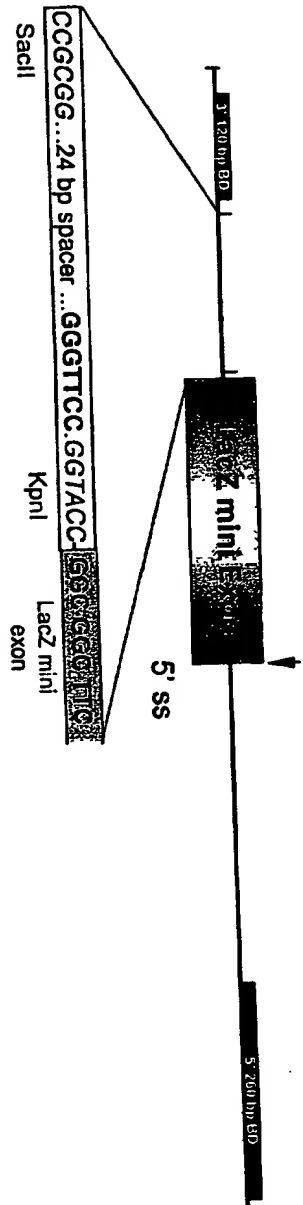
(3) Branch point, pyrimidine tract and acceptor splice site: TACTAAC T GGTAAC T GGTACC TCTTCTTTTTTTT GATATC CTGCAG GGG GGG
BP Kpn I PPT 3'ss LacZ mini exon

(4) 5' donor site and 2nd spacer sequence: GGG GGG GTAAGT GTTATCACCGATATGTGTAACTGATTCTGGCCTTCGATACG
5'ss LacZ mini exon
CTAAGATCCACCGG

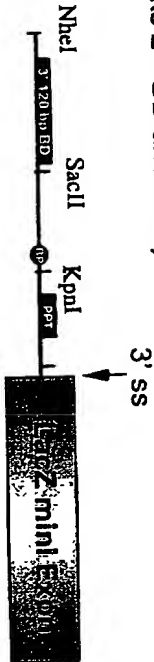
(5) 5' BD (260 BP) : TCAAAAAGTTTTCACATAATTTCTTACCTCTTGAAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCTGGAA
ACACCAATGATTTTTCTTTAATGGCTGCGATAATCCTGGAAACTGATAACACAATGAATCTTCCACTGTGCTTAA
AAAAACCCTCTGAAATTCCTCCATTCTCCCAATAATCATCATTAACAACCTGAACCTCTGGAAATAAAACCCCATCATTAATTAACATCA
TTATCAAAATCAGGC

Figure 21

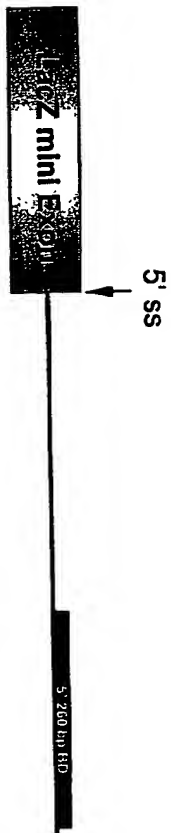
DSPTM8 : (▲ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



Mutants

Figure 22

Double Trans-splicing Produces Full-length Protein

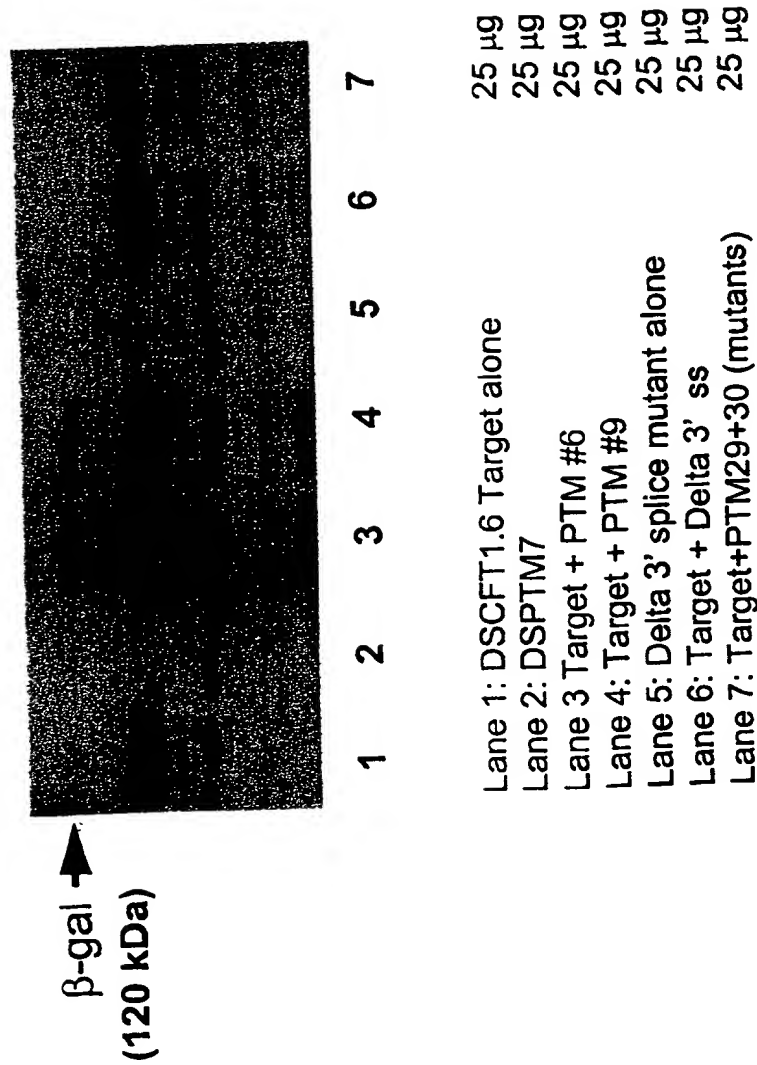


Figure 24

Restoration of β -Gal Function by Double Trans-splicing

Sheet 32 of 58

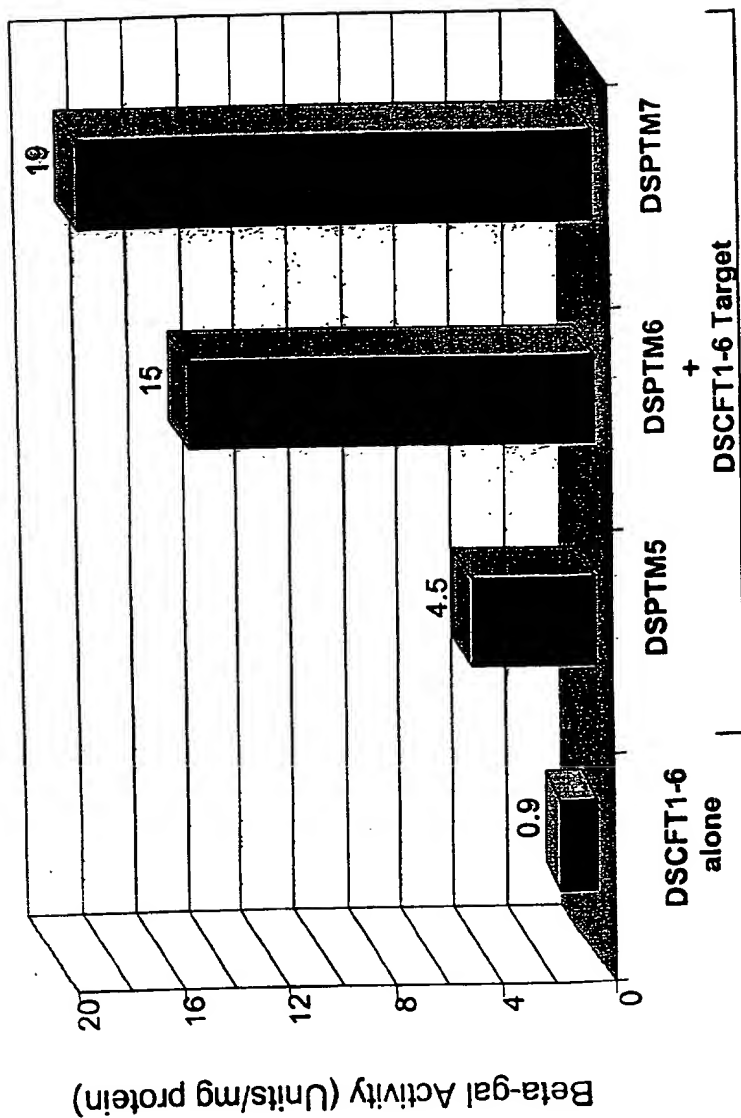


Figure 25

Sheet 33 of 58

Restoration of β -gal activity is due to double RNA trans-splicing events

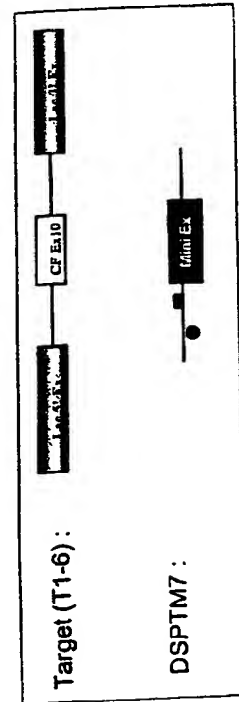
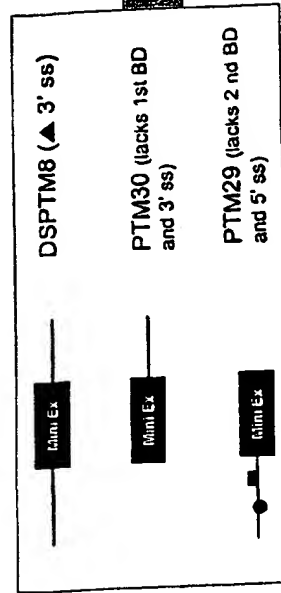
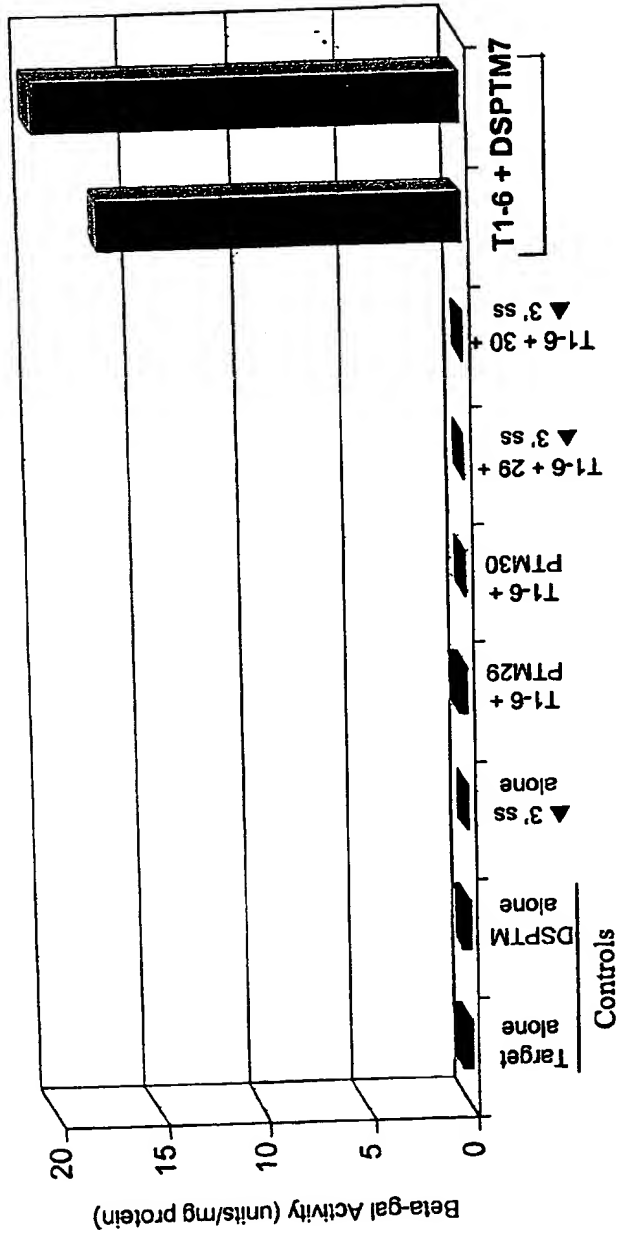


Figure 26

Double Trans-splicing: Titration of Target & PTM

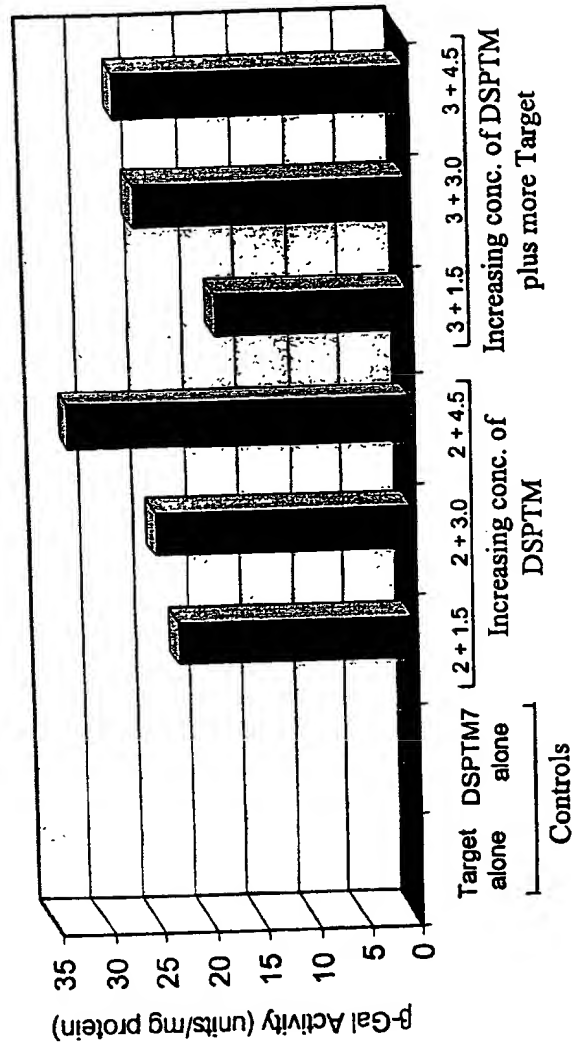
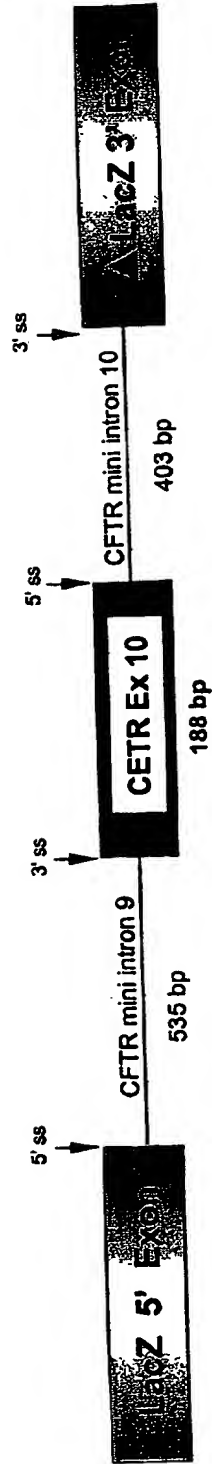


Figure 27

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DSCFT1-6 (Specific Target):



DSHCGT1 (Non-specific Target):

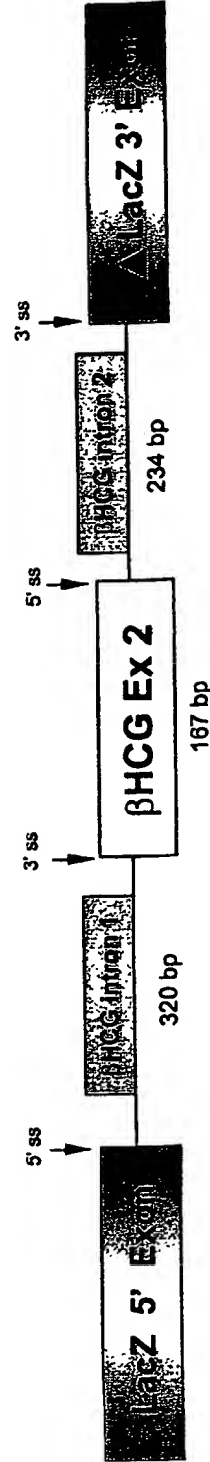


Figure 28

Sheet 36 of 58

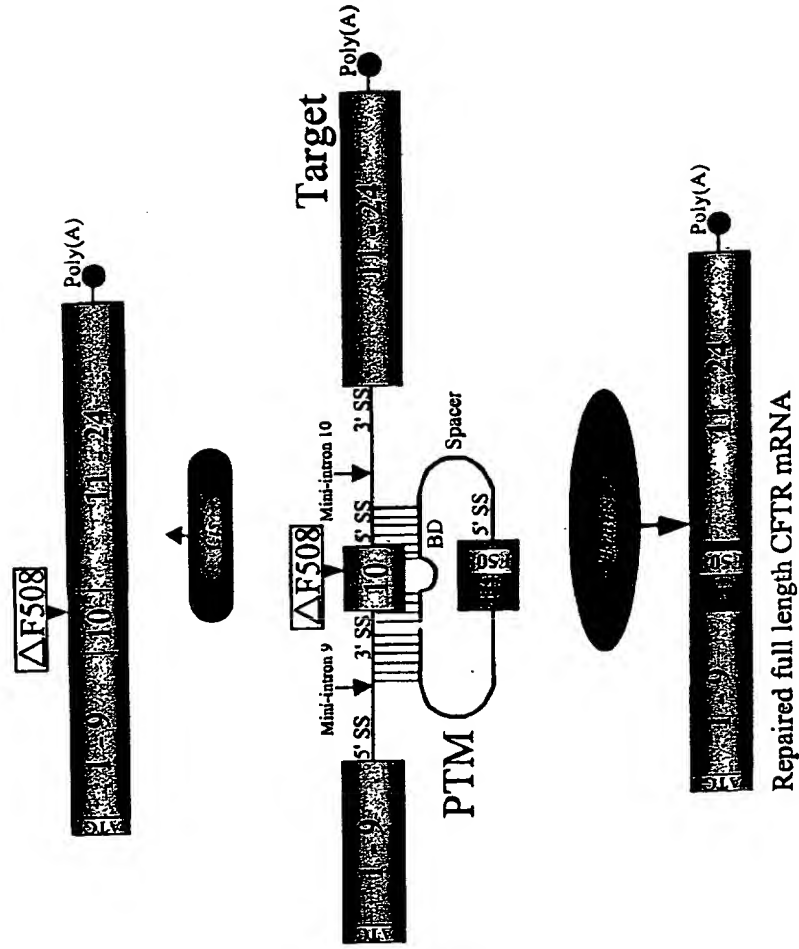
Bar graph showing Beta-gal Activity (units/mg protein) for three conditions:

Condition	Beta-gal Activity (units/mg protein)
DSHCG-T1 alone	1.1
DSHCG-T1 + DSPTM7	1.1
DSCF-T1.6 + DSPTM7	34

Figure 29

Sheet 37 of 58

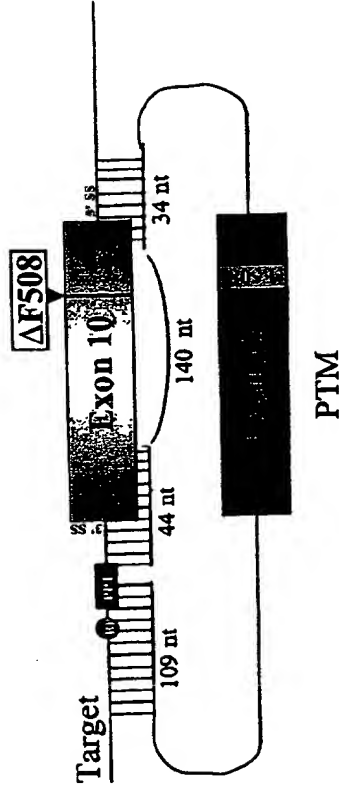
Replacement of a Single Intron with a Spliceosome Diagram of a CFTR Intron



INTRONIN

Figure 30

PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target.



ACGAGCTTGGCTCATGATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAAACATTCCG
 GCCGCATCAGCTTTTGCAGCCAAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAAACATAAT
 CTTCCGCGTCAGTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCCTGTCAGTTGGAGGAG

MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (bold and underlined).

Figure 31

INTRON

Sheet 38 of 58

Sheet 39 of 58

Sequence of a double
trans-spliced product

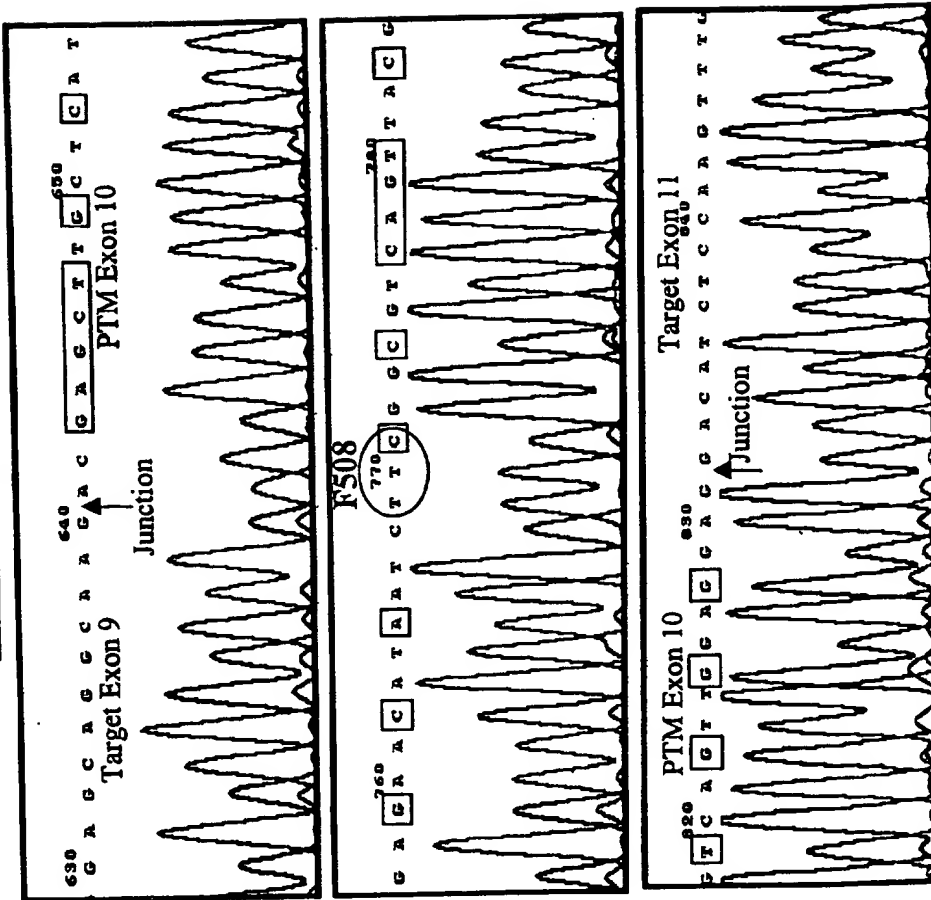


Figure 32

about 40 of 58

CFTR Repair: 5' Exon Replacement

Schematic diagram of a PTM binding to the splice site of intron 10 of an anti-gene target

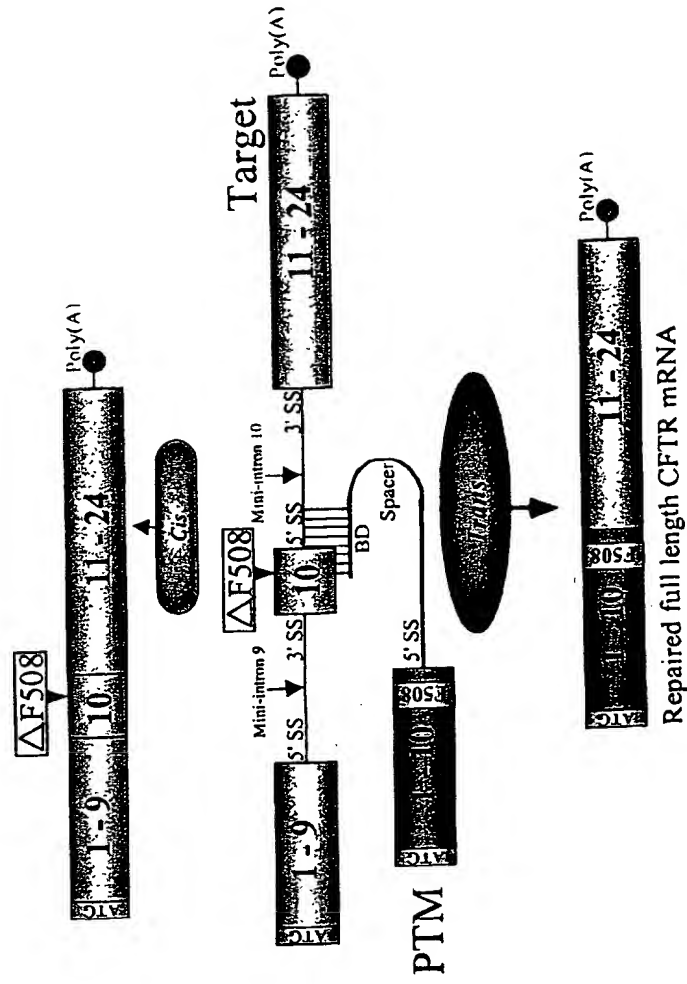
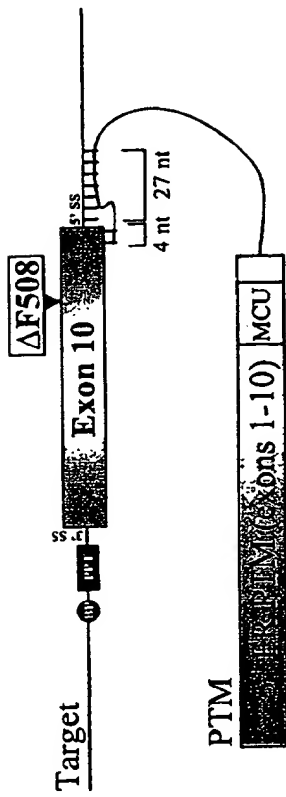
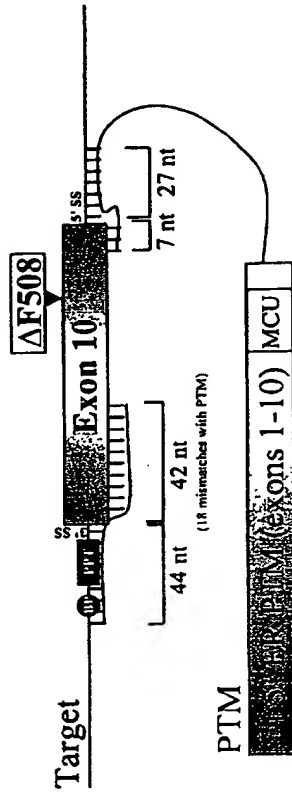


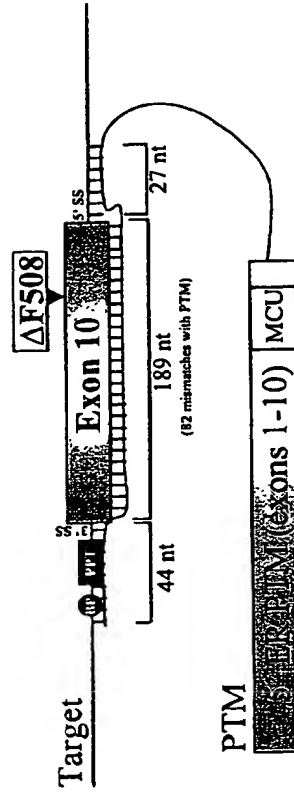
Figure 33



PTM with a short binding domain masking a single splice site in a mini-gene target.

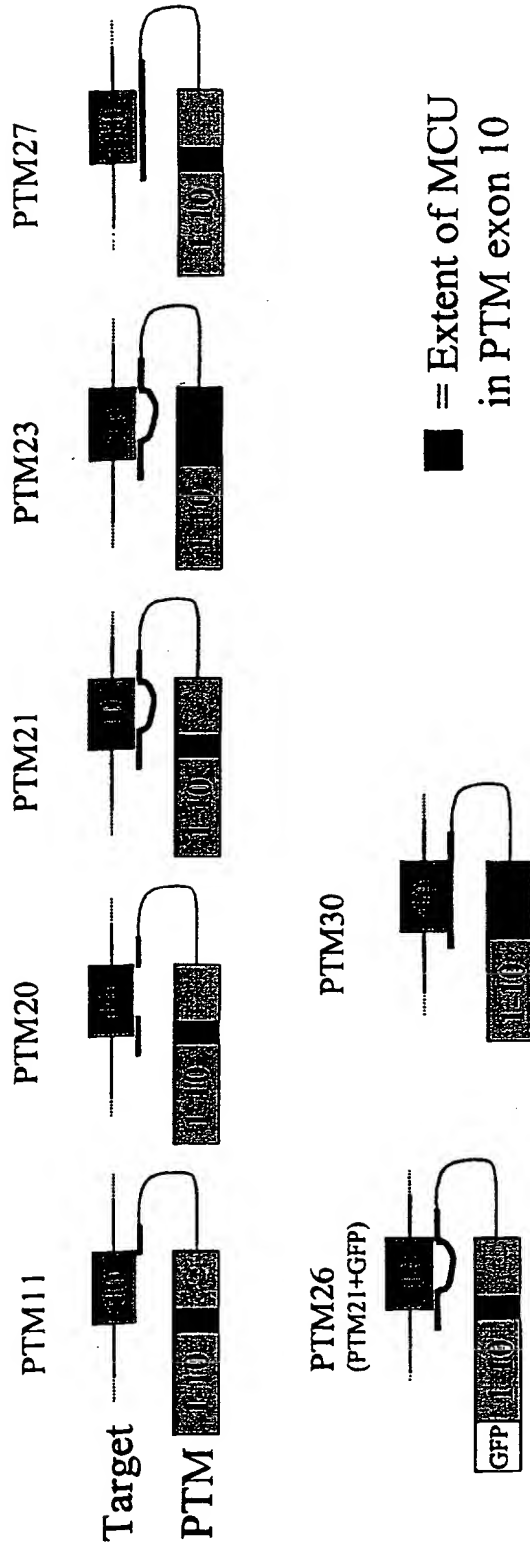


PTM with a long binding domain masking two splice sites in a mini-gene target.



PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

Figure 34



MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCTCATGATGATGGGGAGTTAGAACCAAGTGAAGGCAAGATCAAA~~CATTCCG~~
~~GCCGCATCAGCTTTGCAGCCAA~~TT~~CAGTTGGATCATGCCCGGTACCATCAAGGAGAA~~CATAAT
~~C~~TTCCGGCGT~~CAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAAGGCCTGT~~~~CAGTTGGAGGAG~~

Figure 35

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A

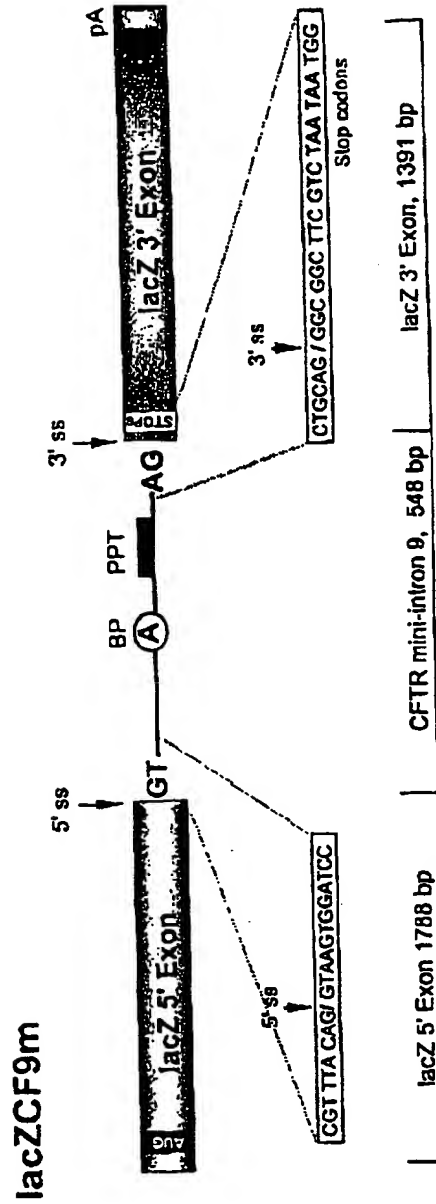


Figure 37 A

$\frac{d}{dt} \left(\frac{\partial L}{\partial \dot{x}} \right) = \frac{\partial L}{\partial x}$

B

Diagram illustrating the structure of the LacZ reporter gene construct. The construct includes a Binding Domain (BD), a Binding Pocket (BP), a Polyoma Pseudotumor Target (PPT), and the LacZ 3' Exon. A 3' splice site (3' ss) is indicated. The construct is 1391 bp long. The sequence is shown below the diagram, with regions labeled N, S, K, and E. The sequence is: GCTAGC (N) - Binding domain (S) - CCGCGG (Spacer) - TACTAAC T (BP) - GGTACC (K) - TCTCTCTTTT (PPT) - GATATC (E) - CTGCAG-GGC GGC (3' ss).



C

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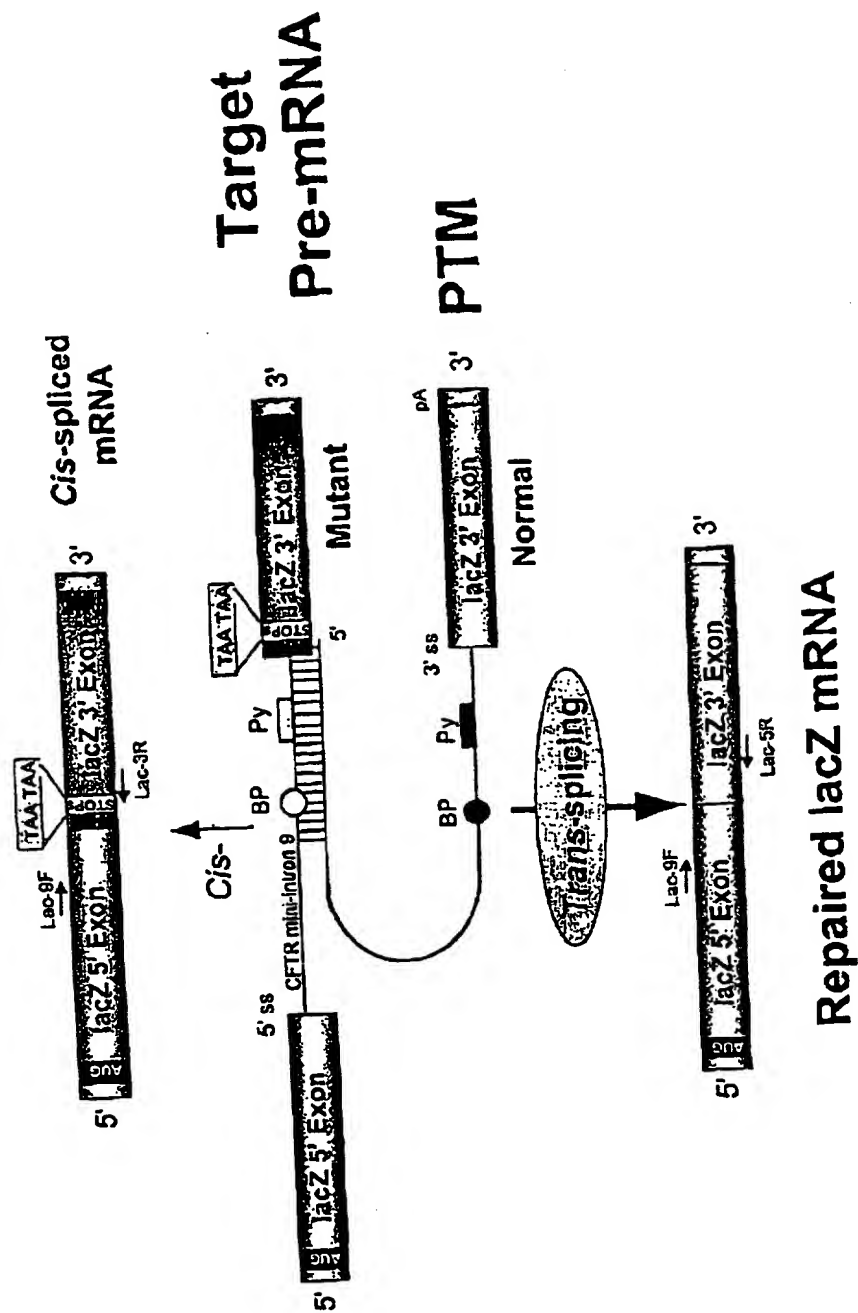


Figure 37C

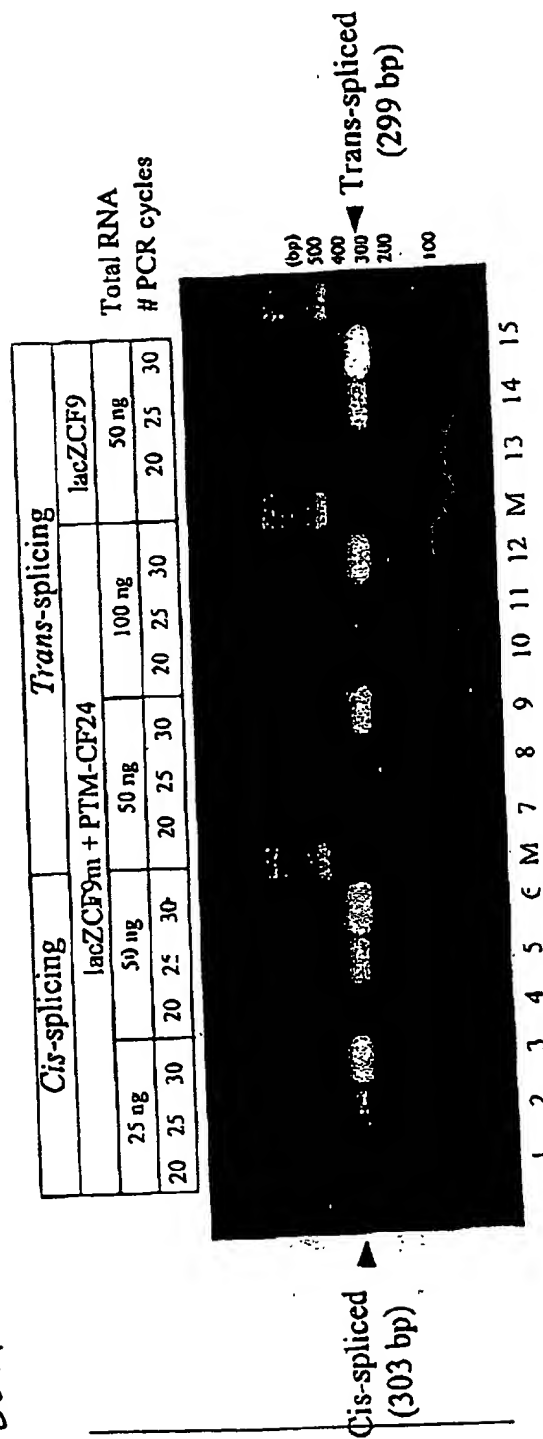
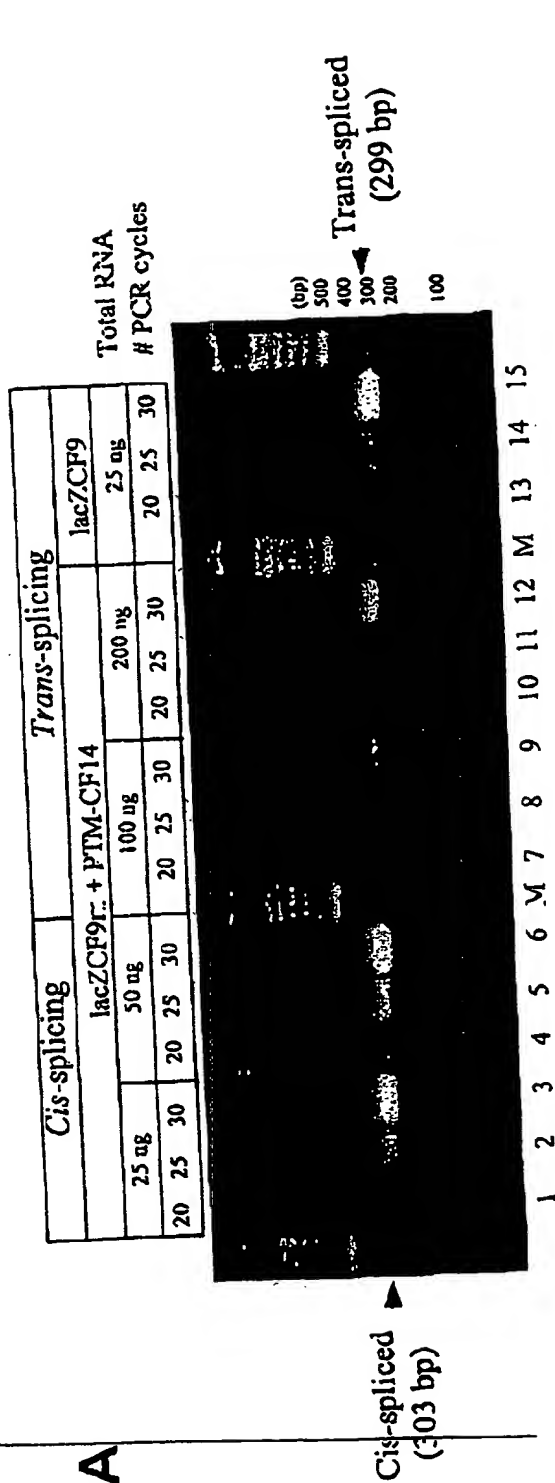


Figure 38 A

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B

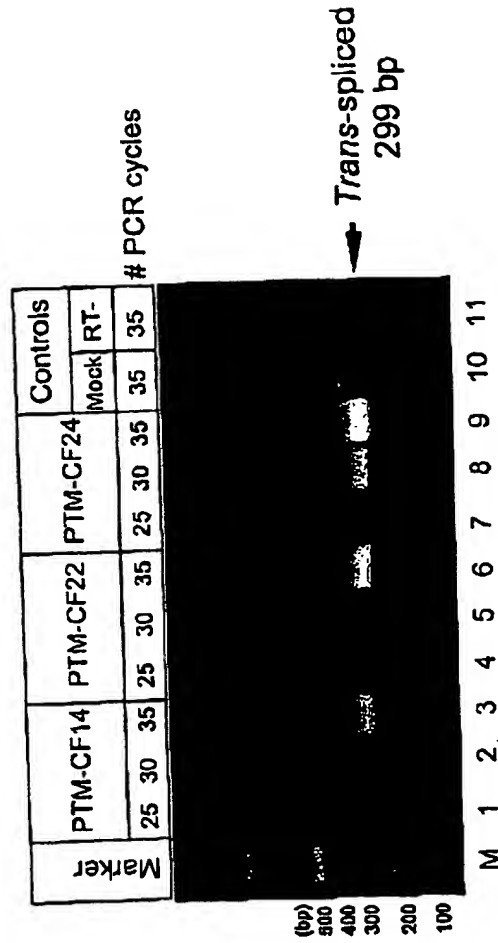


Figure 38B

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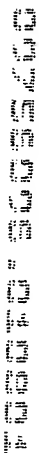
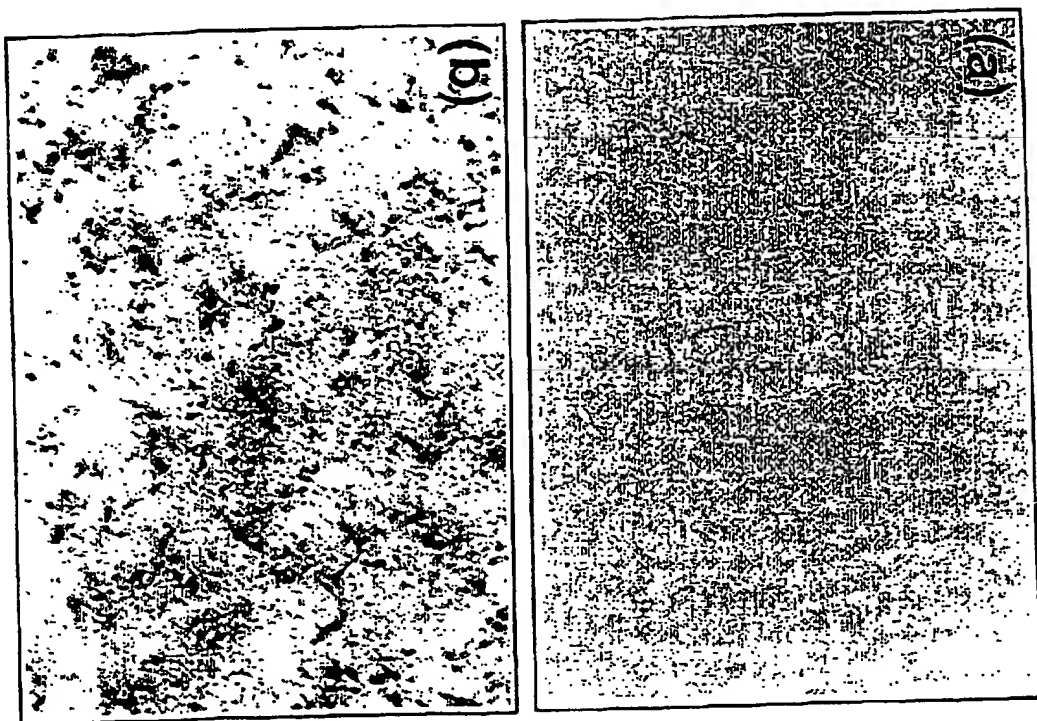


Figure 40A

A

[illegible]

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B

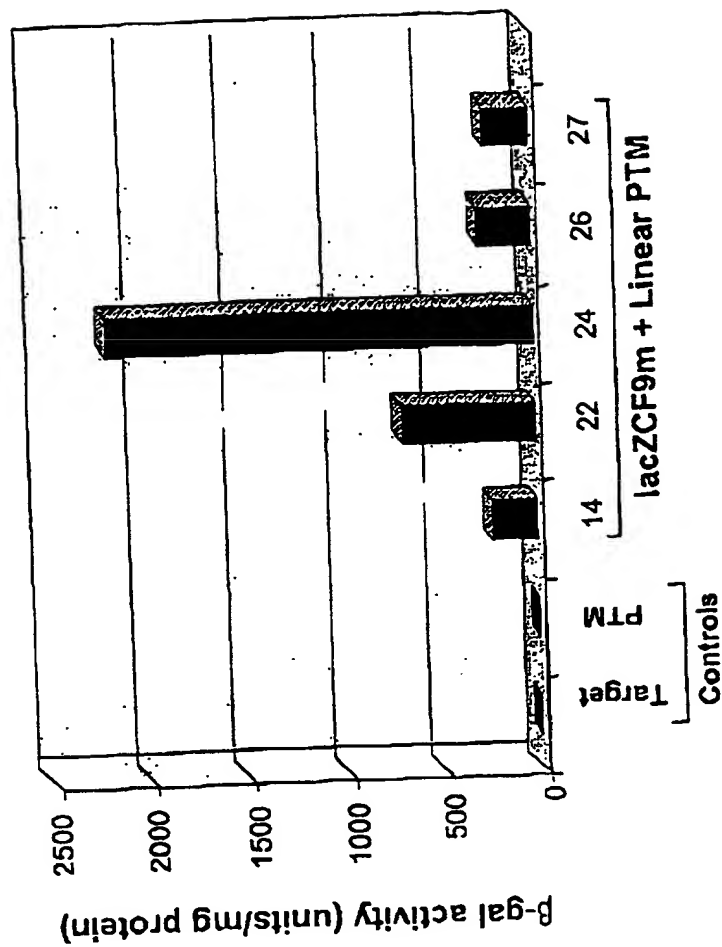


Figure 40B

[illegible]

Condition	β -gal activity (units/mg protein)
Mock	~0.5
Linear PTM	~10
PTM 14	~10
PTM 22	~10
PTM 24	~45

Figure 40C

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A

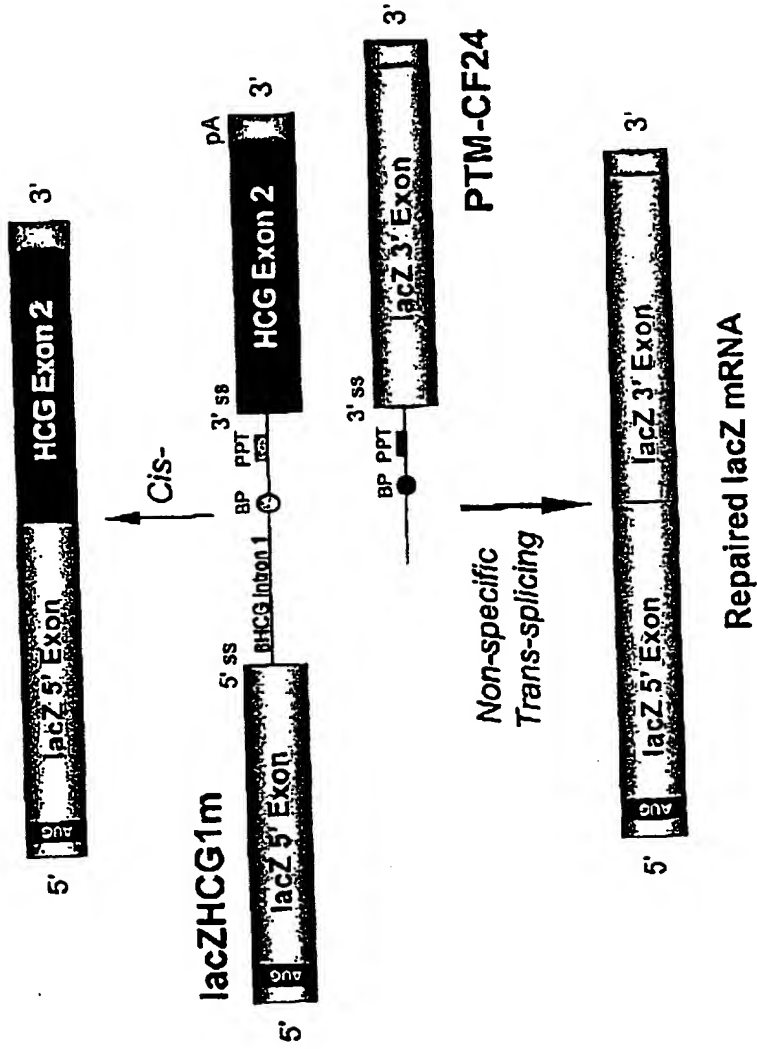


Figure 41A

Sheet 54 of 58

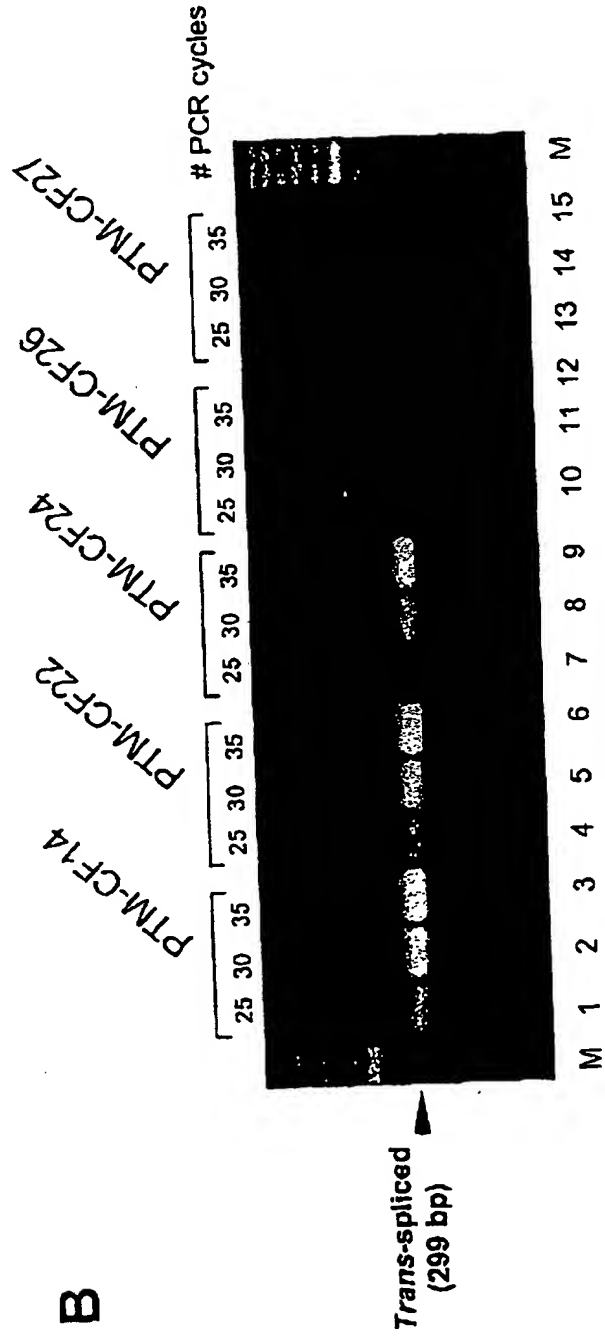


Figure 4KB

Sheet 55 of 58

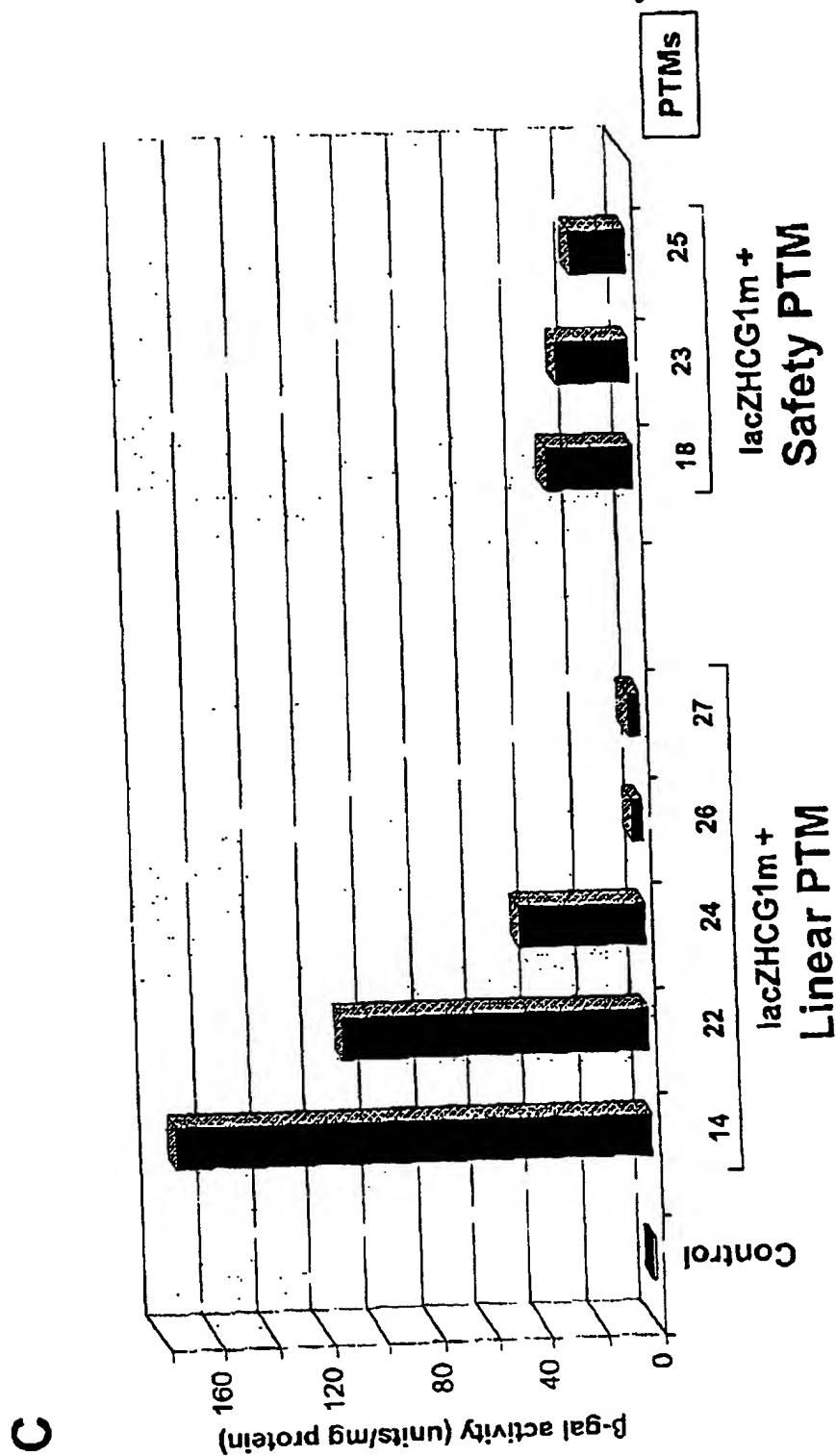


Figure 41C

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Exons 1-10

ATGCAGAGGTCGCCTCTGGAAGGCCAGCGTTGTCTCCAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
 GATACAGACAGCGCCTGGAATTGTCTAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
 GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTTTCTGG
 AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA
 TAGCTTCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTATCTAGGCATAGGCTTATGCCTTCTCTTTAT
 TGTGAGGACACTGCTCTACACCCAGCCATTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT
 TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTTGGACAACCTTGTTAGTCTCCITT
 CCAACACCTGAACAAATTTGATGAAGGACTTGCAATGGCACATTTCTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
 CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCTGTATAGTCCCTGCCCCTTTTTCAG
 GCTGGGCTAGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG
 AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAATGATTGAAAACCTTAAGACA
 AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTCTCAGGGTTCTTT
 GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGAAAAATATTACCCACCATCTCATTCT
 GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA
 CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG
 AATGTAAACAGCCTTCTGGGAGGAGGGATTGCGGAATTATTGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTT
 CTAATGGTGATGACAGCCTCTTCTTCACTAATTTCTCACTTCTTGGTACTCTGTCTGAAAGATATTAATTTCAAGAT
 AGAAAGAGGACAGTTGTTGGCGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTCTCATGATGATCATGGGCGAG
TTAGAACCAAGTGAAGGCAAGATCAACATTCCGGCCGCATCAGCTTTTGACGCCAATTCACTTGGATCATGCCCGTA
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAAGCCTGTCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTGGGCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCCTCCACTGT
GCTTAATTTTACCCTCTGAATTTCTCCATTTCTCCATAATCATCATTACAACCTGAACCTCTGGAAATAAAACCATCATT
ATTAACCTCATTATCAATCACGCT

Figure 42

[illegible]

Nhe I

153 bp BD underlined

GCTAGC-GACGAAGCCGCCCTCAGCTCAGGATTCACTTGCCCTCCAAATTATCATCTAAGCAGAAGTGTTATA

TTCTTATTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTAAATACTTCCGTGTTTCACTACTCTGCTATGC

Sac II

AC-CCGCGG

Figure 43A

Sheet 58 of 58

Trans-splicing domain

AATAATGACGAAGCCGCCCTCACGCTCAGGATTCACTTGCCCTCCAATTATCATCCTAAGCAGAAGTGATATTTCTTA
TTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAATACTTCTGTTTCACCTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTGCTCGAATACTAAGTGGTACCTCTTCTTTTTTTTTTGATATCCTGCAG

Exons 10-24

ACTTCACCTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAAGAATTTCACTTCT
GTTCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTTCTATGATGAATATAGATA
CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGAGAA
GGTGGAAATCACACTGAGTGGAGGTCAACGAGCAAGAAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTGTATT
TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTGAAAGCTGTGTCTGTAACTGATGGC
TAACAAAACCTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTGTCATGAAGGT
AGCAGCTATTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAAAACCTCATGGGATGTGATT
CTTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCATCTTAAGTGGAGCCTTACACCGTTTCTCATTAGAAGGAGATGC
TCCTGTCTCCTGGACAGAAACAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAGGAAGAATTTCTATT
CTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAAAAGACTCCCTTACAAAATGAATGGCATCGAAGAGGATT
CTGATGAGCCTTTAGAGAGAAGGCTGTCTTAGTACCAGATTCTGAGCAGGGAGAGGCGATACTGCCTCGCATCAGCGT
GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGAGTCTGTCTGAACCTGATGACACACTCAGTTAACCAAGGT
CAGAACATTCACCGAAAGACAAACAGCATCCACACGAAAAGTGTCACTGGCCCTCAGGCAAACTTGACTGAACCTGGATA
TATATTCAAGAAGGTATCTCAAGAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT
TTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCACAGAGCTTA
ATTTTGTGCTAATTTGGTGCTTAGTAATTTTCTGGCAGAGGTGGCTCTTCTTGGTTGTGCTGTGGCTCCTTGGAA
ACACTCCTCTCAAGACAAAGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC
GTATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCACTGGTG
CATACTCTAATCACAGTGTGCAAAAATTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCTCA
ACAGTTTGAAGCAGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTTACCAT
ATTTGACTTCATCCAGTTGTTATTAATTTGTGATTGGAGCTATAGCAGTTGTGCGAGTTTACAACCTTACATCTTTGTT
GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCTCCAAACCTCAGCAACTCAAACAACCTGG
AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTCGGAGC
GCAGCCTTACTTTGAAACTCTGTTCCACAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTAACCTGTCAACACTG
CGCTGGTTCCAAATGAGAATAGAAATGATTTTGTGATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAAACAG
GAGAAGGAGAAGGAAGATTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGGCTGTAACTC
CAGCATAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTAAAGTTCAATGACATGCCAACAGAAGGTAAACCT
ACCAAGTCAACCAACCATAACAAGATGGCCAACCTCTCGAAAGTTATGATTATTGAGAATTACACGTGAAGAAAGATG
ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAATGCCATATTAGA
GAACATTTCTTCTCAATAAGTCTTGCCAGAGGGTGGCCCTCTTGGGAAGAACTGGATCAGGGAGAGTACTTTGTTA
TCAGCTTTTTTGGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGCAAC
AGTGGAGGAAAGCCTTTGGAGTGATACACAGAAAGTATTTATTTTTCTGGAACATTTAGAAAAAAGCTTGGATCCCTA
TGAACAGTGGAGTGATCAAGAAATATGGAAGTTGCGAGATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCTGGG
AAGCTTGACTTTGTCTTGTGGATGGGGGCTGTGCTTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
TTCTCAGTAAGGCGAAGATCTGTGCTTGATGAACCCAGTGCTCATTGGATCCAGTAACATACCAAATAATTAGAAG
AACTCTAAAAACAAGCATTTGCTGATTGCACAGTAATTTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
TTTTTGGTATAGAAGAGAACAAAGTGGCAGTACGATTTCCATCCAGAACTGCTGAACGAGAGGAGCCTTTCCGGC
AAGCCATCAGCCCTCCGACAGGGTGAAGCTCTTCCCAACCGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATTGC

Histidine tag Stop

TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATATTAG

Figure 43B